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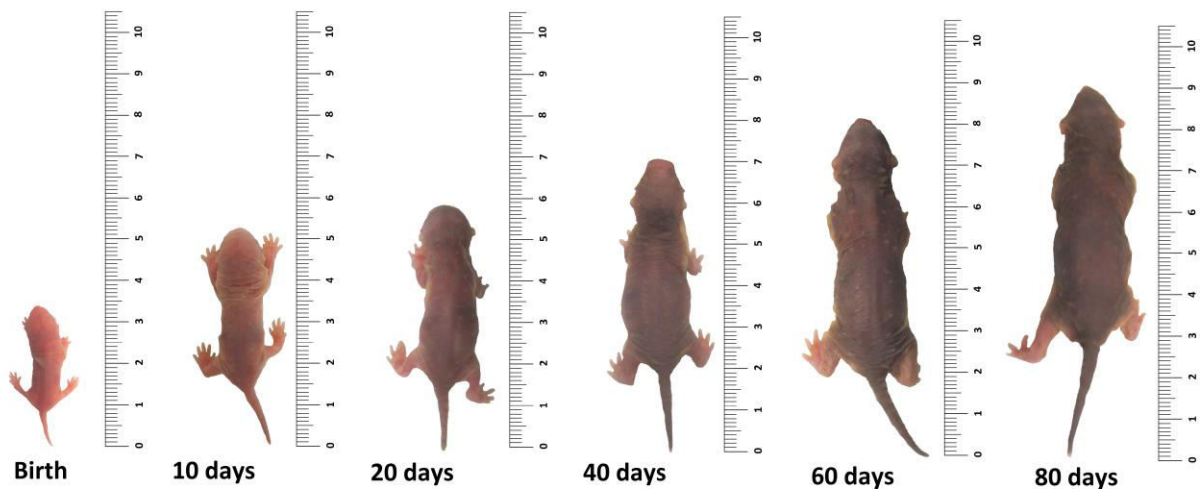
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Michaela Morhart



I am concerned here with some animal unknown to me.

That is possible.

True, I have observed the life down here long and carefully enough,
but the world is full of diversity and is never wanting in painful surprises.

Yet it cannot be a single animal,
it must be a whole swarm that has suddenly fallen upon my domain,
a huge swarm of little creatures.

Franz Kafka, *The Burrow*

Für Harry Henle

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Abbreviations

NMR	Naked mole-rat
IZW	Leibniz Institute for Zoo and Wildlife Research
hr	hour
d	day
wk	week
mo	month
yr	year
mm	millimetre
mm/wk	millimetre/week
mm/yr	millimetre/year
cm	centimetre
m	metre
km	kilometre
m ²	square metre
g	gramme
g/d	gramme/day
kg	kilogramme
kg/hr	kilogramme/hour
ml	millilitre
l/ml	litre/millilitre
°C	degree Celsius
%	per cent
Ø	average
±	Standard deviation
am	ante meridian (before noon)
pm	post meridian (after noon)

Summary

The majority of subterranean rodent species exhibits a complex social system related to their specific reproductive organization. Therefore they are often difficult to breed in captivity.

This holds true for the eusocial naked mole-rat, *Heterocephalus glaber* (Rüppell 1842), which is being kept in many zoos and institutes worldwide, but successful breeding and rearing of offspring are also rare. Naked mole-rats (NMR) live in colonies that may contain up to 300 individuals; reproduction is monopolised by a single dominant female the so-called “queen”, and one or two males. Their social system is further characterised by a division of labour, overlapping generations and co-operative care for the offspring. The progeny usually remains in the natal burrows and - in established colonies - benefits from the co-operative foraging activities of the adult individuals of the colony.

The aim of this master thesis was to survey selective aspects of pup ontogeny and reproduction in two captive naked mole-rat colonies at the Leibniz Institute for Zoo and Wildlife Research (IZW) in Berlin. During the last four years (2008-2012), three queens of two colonies gave birth to a total of 273 pups in 23 litters, with a mean litter size of 11.87 ± 3.68 pups. The average inter-birth interval was 103.55 ± 39.37 days (d). Sex ratio at birth showed slightly higher numbers of males $56 \pm 13.21\%$, continued to be male skewed throughout all age classes. Mean birth weight of the neonates was 1.90 ± 0.20 g. Our study showed that newborns with a birth weight under 1.40 g did not survive the first days of their life. All pups lost body weight in their postnatal days, but in general they regain their birth weight at the 5th day. Female pups weighted significantly less than their brothers at birth (t-Test, $n=243$, $t=0.66$, $p=0.02$). In general, female pups gain body weight faster within the first 80 d postpartum, compared to their male littermates. The captive born pups of the IZW, both male and female, showed an extremely low growth rate in comparison to other subterranean rodents. Interestingly, the pups exhibited a wide variation of individual body weight within and between litters throughout their lifespan. Even though pup mortality is generally high in captive NMR colonies, on average 42% of the offspring survived in the study colony. Pups who reached an age of three months, which is at the end of the weaning period, survived in 99% of the cases. Surprisingly, only 46% of the dead newborns could be retrieved, the remaining were probably consumed by the colony. The

colony members did not only feed on dead pups, as it was observed that two of three queens ate pups which were still living.

The present results may contribute to a better understanding on the development of the complex social system of NMR and its functions, as well as they may help to enhance the breeding success of NMR colonies in captivity.

Zusammenfassung

Eine Vielzahl von unterirdisch lebenden Nagetierarten weist eine komplexe Sozialstruktur hinsichtlich ihrer Fortpflanzung auf, weshalb sie nur schwer in Gefangenschaft zu züchten sind.

Dies trifft insbesondere auf den eusozialen Nacktmull, *Heterocephalus glaber* (Rüppell 1842) zu, welcher in vielen Zoos und Instituten weltweit gehalten wird. Auch bei dieser Art gelingt die Nachzucht so wie das erfolgreiche Aufziehen der Jungtiere nur selten.

Nacktmulle leben in großen Kolonien mit Populationsgrößen von bis zu 300 Individuen, jedoch ist die Fortpflanzung allein einem dominanten Weibchen, der sogenannten „Königin“, mit ein bis zwei Männchen vorbehalten. Das Sozialsystem von Nacktmullen ist charakterisiert durch Arbeitsteilung, überlappende Generationen und eine kooperative Bruthilfe. In der Regel verbleibt der Nachwuchs stets in den Bauten, in denen er geboren wurde und profitiert so vor allem in bereits etablierten Kolonien von der kooperativen Nahrungssuche durch ältere Kolonienmitglieder.

Ziel der Masterarbeit war es, ausgewählte Aspekte der juvenilen Individualentwicklung und Fortpflanzung von zwei in Gefangenschaft lebenden Nacktmullkolonien zu erforschen. Hierfür wurden die erfassten Daten von einer Untersuchungsperiode von vier Jahren (2008-2012) von zwei reproduktionsaktiven Nacktmullkolonien des Leibniz-Institutes für Zoo- und Wildtierforschung (IZW) in Berlin ausgewertet.

In diesem Untersuchungszeitraum haben drei Königinnen aus den beiden Kolonien insgesamt 273 Jungtiere aus 23 Würfen geboren, mit einer mittleren Wurfgröße von $11,87 \pm 3,68$ Neugeborenen. Im Durchschnitt ergab das Intervall zwischen zwei Geburten $103,55 \pm 39,37$ Tage. Bei der Geburt betrug das Geschlechterverhältnis $56 \pm 13,21\%$ und war leicht zugunsten der Männchen verschoben. Dieses Geschlechterverhältnis blieb nahezu konstant über alle Altersklassen bestehen. Die Neugeborenen kamen mit einem durchschnittlichen Geburtsgewicht von $1,90 \pm 0,20$ g zur Welt, wobei die weiblichen Nachkommen signifikant (t-Test, $n=243$, $t=0.66$, $p=0.02$) weniger bei der Geburt wogen als ihre Brüder. Die Studie zeigte auch dass Jungtiere mit einem Geburtsgewicht unter 1,40 g nicht überlebten. In den ersten Tagen nach der Geburt verloren alle Neugeborenen zunächst an Gewicht, jedoch erreichten sie in der Mehrzahl der Fälle ihr Geburtsgewicht wieder am Tag 5 nach der Geburt. Alle Jungtiere, welche im Rahmen dieses Forschungsprojektes am Institut geboren wurden, wiesen im

Vergleich zu anderen unterirdisch lebenden Nagern ein langsames Größenwachstum auf. Jedoch legten die weiblichen Jungtiere, im Vergleich zu ihren männlichen Wurfgeschwistern, innerhalb der ersten 80 Tage nach der Geburt noch etwas mehr an Gewicht zu. Dabei zeigten sich auch große Variationen bezüglich der individuellen Körpermasse innerhalb eines Wurfes wie auch zwischen einzelnen Würfen durch alle Altersklassen hinweg. Aufgrund einer allgemein hohen Kindersterblichkeit bei in Gefangenschaft gehaltenen Nacktmullkolonien, überlebten in den untersuchten Kolonien im Durchschnitt nur 42% der Jungtiere eines Wurfes. Eine Überlebenswahrscheinlichkeit von 99% hatten Jungtiere, wenn sie 3 Monate und somit die Entwöhnungsphase von der Mutter überlebt hatten. In der Regel konnten noch 46% der toten Jungtiere wieder aufgefunden werden, die Verbliebenen wurden wahrscheinlich von Individuen der Kolonie gefressen. Hierbei wurden nicht nur tote Nachkommen von der Kolonie aufgefressen, sondern es wurde auch beobachtet, wie zwei Königinnen lebendige Neugeborene verzehrten. Die gewonnenen Erkenntnisse über das Geschlechterverhältnis und das Wachstum der Jungtiere in dieser Studie leisten einen wichtigen Beitrag zum Verständnis für das komplexe Sozialsystem der Nacktmulle, insbesondere dem Kastensystem mit seiner Arbeitsteilung, wie es sich möglicherweise aufbaut, entwickelt und funktioniert. Des Weiteren könnte das erworbene Wissen über die Reproduktion (Geburtsintervalle, Wurfgröße, etc.) bei Nacktmullen dabei helfen, den Fortpflanzungserfolg und das erfolgreiche Aufziehen von Jungtieren in Gefangenschaft zu verbessern.

1. Introduction

The aim of this thesis was to study selective aspects of pup ontogeny and reproduction in two captive NMR (*Heterocephalus glaber*, Rüppell 1842) colonies.

The NMR belong to the Bathyergidae family of subterranean rodents endemic to Africa, which includes six genera and 22 species (Ingram et al. 2004; Kock et al. 2006). Three of these genera are strictly solitary (*Georychus*, *Bathyergus* and *Heliophobius*), while the other three are social (*Cryptomys*, *Fukomys* and *Heterocephalus*; Burda et al. 2000). The three social genera are of particular scientific interest regarding their reproduction and pup ontogeny, being the only eusocial genera known to date (Jarvis 1981; Jarvis and Bennett 1993). They exhibit a colony structure similar to that of eusocial ants, termites and bees (Wilson 1971). Their social system is characterised by the monopolisation of reproduction by a single female, the “queen”, which together with one to three males produces the entire offspring of a colony (Faulkes et al. 1997). Other remarkable features of NMR colonies are division of labour, overlapping generations and co-operative care for the offspring. The pups of social mole-rats grow up and remain in their natal colony for their entire lives. This may explain their extremely low growth rates and slow development compared to other non-bathyergid and solitary surf dwelling or subterranean rodents, which usually have to disperse soon after birth (Bennett et al. 1991).

Due to the fact that NMR live in deep, inaccessible large and subterranean burrows (Brett 1991), observing and catching in the wild nature has proven to be very difficult (Bennett et al. 1991; O’Riain and Jarvis 1997). Furthermore, it is extremely challenging to successfully breed bathyergid species in captivity (Bennett et al. 1991; Hood et al. 2014; Ke et al. 2014).

Within the bathyergid genera the breeding in captivity of the social genera - such as the NMR - is known to be easier than breeding the solitary ones (Bennett et al. 1991). However, even rearing offspring of the social species often is difficult and success is infrequent. In comparison to the NMR, most other bathyergid species produce smaller litters (1 to 10 newborns; Bennett et al. 1991), particularly the solitary ones. Of all mole-rat species, the NMR produces the largest litters (1 to 30 pups; R. Buffenstein, pers. comm. 2013). Generally, in larger litters the parental investment for each individual is usually lower than in smaller ones, resulting in increased pup mortality. This increased mortality may be explained by a higher competition between the

siblings, e.g. for access to the teats during lactation and for obtaining the best topographical advantages (i.e. the best position for suckling).

Due to the problems in regards to reproduction mentioned above for many species of NMR data on reproduction and pup ontogeny, e.g. birth weight, gender and development are incomplete and are mostly based on a small number of publications with reliable data (Bennett et al. 1991; Jarvis and Bennett 1991).

Though NMR are kept in many zoos and research institutes worldwide, successful breeding is rare (Hood et al. 2014; Ke et al. 2014) and even for these cases, studies including information on pup ontogeny and development are scarce and based on limited reliable data (Bennett et al. 1991; Jarvis 1991; O'Riain 1996). The first study about reproduction and pup ontogeny in the NMR was published by Jarvis (1991), who tried to address this lack of knowledge. However, her study did not comprise large litters and long-term observations on a daily basis. Several years later, O'Riain (1996) provided the first detailed long-term study on the behavioral and morphological ontogeny of captive NMR from birth to adulthood. The study is limited, as it is based on a small sample size of 40 pups from two pairs (6 litters), with a maximum litter size of 13 newborns. Another shortcoming is that O'Riain (1996) started collecting most of his data after weaning, when the pups were already about 40 d old, as he wanted to avoid an increased pup mortality due to stress caused by handling the newborns; it is known that frequent disturbances, e.g. by handling or loud noise during the first weeks after birth often result in the death of the pups (Jarvis 1991). Consequently, O'Riain did not obtain individual behavioral profiles prior to weaning. The aim of this master thesis was to close this gap of knowledge and assess diverse parameters of the early ontogeny of NMR in greater detail.

In this thesis data of selective traits of reproduction and pup ontogeny in captive NMR are included. The data were collected from two of the most successfully breeding colonies throughout Europe, kept at the IZW. The high number of newborns (sample size: n=273 newborns; 23 litters) and a well-trained pup-handling method provided the possibility to conduct the first detailed study about selective traits of reproduction and ontogeny on a daily basis from birth on. Furthermore, for the first time the sex ratio of the newborns and the gender of the pups with regard to birth weight, growth development and mortality rate were analysed in this study.

These data represent an important asset to previous studies and gives new insights into the functioning of the social system of NMR, e.g. the influence of gender of a pup on its survival, growth and development. The findings of this study may also prove useful for investigating the still open question on the existence of a gender-linked division of labour. In addition, the presented data of NMR pups may provide a good basis for comparisons with the other mole-rat species, particularly with the social genera of *Cryptomys* (Bennett and Jarvis 1988) which display a very similar colony structure, regarding the pup rearing and their development.

1.1 Literature review

In the mid-1970s, Richard Dean Alexander, an evolutionary biologist at the University of Michigan attempted to explain the apparent absence of eusociality in mammals. Eusociality is characterised by division of labour, reproductive castes and, cooperative brood care, with more or less sterile members and overlapping generations between mothers and their adult offspring (Batra 1966; Michener 1969; Wilson 1971). At this time no eusocial mammal had been identified (Alexander et al. 1991). This complex social structure was only observed in insects such as termites, bees, ants and wasps. In his lecture Alexander philosophised about the hypothetical setting for the existence of an eusocial mammal, postulating by a fabulous animal with all the characteristics needed for eusociality: (i) a strictly subterranean rodent, (ii) living in burrows with a safe communal nest and (iii) inaccessible to most predators in a hot, dry and humid habitat with heavy mud soil and (iv) exploiting an almost safe and large food source (Alexander et al. 1991).

Unknown to R.D. Alexander at that time, he had given an almost complete description of the NMR (*Heterocephalus glaber*; Fig. 1). This really existing extraordinary and unique animal is a bathyergid rodent living in deep, subterranean burrows in the Horn of Africa and resembles in many ways more a social insect than a mammal.



Fig. 1: Wild naked mole-rat in Ethiopia.

1.2 Naked mole-rats history and biology

The first comprehensive description of the NMR has been compiled by the German natural scientist Eduard Rüppell in the year 1842 in the course of his studies documenting the African mammals (Rüppell 1845).

His NMR exemplar originated from Schoa southern Abyssinia (Ethiopia) and was provided by Martin Bretzka, an old expedition companion of Rüppell. Rüppell aptly named this animal “different-headed” (*Heterocephalus*) and smooth-skinned/hairless (*glaber*) because of its callow and hairless skull (Fig. 1). Initially Rüppell didn’t know what was presented to him - either a baby of a larger pilary adult or an ill animal that had lost his complete coat as part of pathology. After the collection of plurally individuals of these subterranean rodents by Bottego, Parona, Cattaneo and co-authors (Brett 1986; Jarvis and Bennett 1991), this question was definitively answered in 1893. Since that time, NMR were recognised as an own species.

The NMR is one of the most extraordinary mammals and exceptional in a large number of aspects. One of their special characteristics is the high life expectancy. Generally, the body mass correlates with the maximum lifespan of an animal (Fig. 2). *H. glaber* is comparable to *Mus musculus* with a size of ~15 cm and body weight ~35 g. However, their maximum lifespan is ~32 years (yr) in captivity (Buffenstein 2008), this is about seven times longer than the maximum captive life span of a laboratory mouse (Prothero and Jurgens 1987).

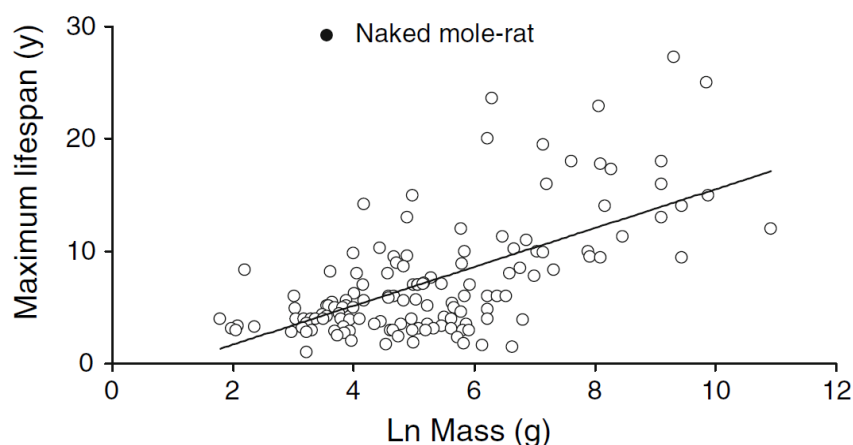


Fig. 2: Plot of the relationship between maximum longevity and log-transformed adult body weight for rodents (Buffenstein 2008).

NMR do not have the life expectancy observed in most rodents as their mortality rates do not correlate with age (Lewis et al. 2012). During their long lifespan, they experience very few of the common characteristic changes associated with age: they largely maintain their body composition, bone density and metabolic rate (Buffenstein 2005). Spontaneous neoplasm has also never been observed in these rodents (Seluanov et al. 2009; Lewis et al. 2012).

NMR are one of only two known eusocial vertebrate species, together with the Damaraland mole-rat (*Fukomys damarensis*; Jarvis 1981; Brett 1991; Jarvis and Bennett 1993; Crespi and Yanega 1995; Bennett and Faulkes 2000). NMR exhibit the most highly social living form of cooperative breeding so far observed in a mammal. A colony typically contains overlapping generations with 70 to 80 individuals, but can sometimes increase up to 300 individuals (Brett 1991a). Similar to eusocial Isoptera (termites; Wilson 1971) and Hymenoptera (wasps, ants and bees) there is a distinct reproductive hierarchy and division of labour within the NMR colonies (Jarvis 1981; Fig. 3).

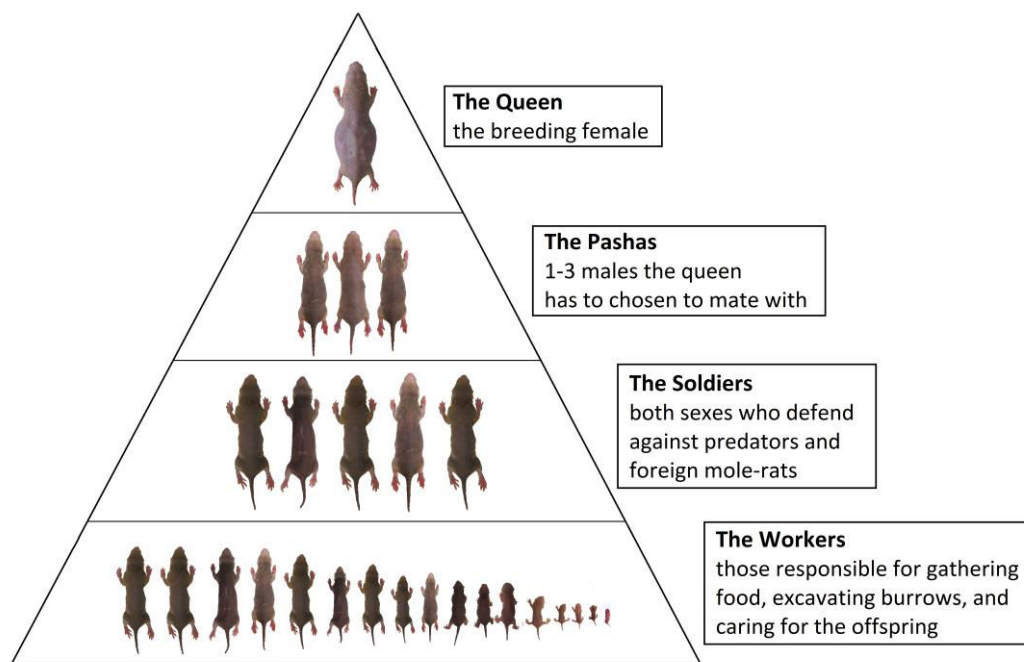


Fig. 3: Division of labour in naked mole-rat colonies.

Each colony consists of a breeding female, the “queen”, one to three breeding males (Jarvis 1981; Lacey and Sherman 1991), workers (Jarvis 1981; Brett 1991; Lacey et al. 1991; Lacey and Sherman 1991) and dispersers (O’Riain et al. 1996; Braude and Ciszek 1998; Braude 2000; Braude et al. 2001; Fig. 3). In contrast to the breeding males, the task of the queen consists not

only in reproduction of all the offspring of the colony. She also is responsible for the sexual suppression of her workers of both genders (Reeve and Sherman 1991), arouse the work activities (Reeve 1992) and nursing her newborns. The queen is known to be the most aggressive and active member of a colony (Lacey and Sherman 1991; Reeve and Sherman 1991; Clarke and Faulkes 2001). All of the other members are non-breeding workers, performing tasks related to burrow excavation, building nests and maintaining the tunnel system (Jarvis et al. 1991; Lacey and Sherman 1991), foraging for subterranean tubers and bulbs. Being also responsible for the offspring care they huddle and groom with them in the nest, retrieve them if they wander out of the nest e.g. during disturbance, and provide fecal pellets for them (Lacey and Sherman 1991, 1997). The soldiers are responsible for the defense of the colony and the burrow against predators e.g. snakes or invaders from foreign colonies (Jarvis et al. 1991; Lacey and Sherman 1991). The role of the dispersers has not been thoroughly investigated yet, due to their rare occurrence in the wild and captivity (Braude and Ciszek 1998; Braude et al. 2001). It is assumed that these animals leave their natal colony to reproduce with other NMR to facilitate the genetic exchange between the colonies (Braude 2000). However, the average genetic relatedness within NMR colonies is the highest of any mammal ($r=0.81$ in Mtito Andei Kenya, compared with 0.5 in siblings of an outbred species; Reeve et al. 1990). Early microsatellite studies of wild NMR colonies point out that over 80% of all copulations take place between first-degree relatives (Reeve et al. 1990). This degree of relatedness is comparable with monozygotic twins or sibling-mated laboratory reared mice after 60 generations (Reeve et al. 1990; Honeycutt et al. 1991b). Altogether, it seems that the costs of dispersal outbalance the costs of inbreeding (Faulkes et al. 1990; Jarvis et al. 1994). Yet, morphological disparities (e.g. skin color, body weight, body size) are discernible even among siblings and determine their individual roles in the group (Jarvis et al. 1991). However, these morphological variations are probably due to epigenetic mechanisms that have not yet been studied, which may also contribute to an observed variety in longevity of the different casts in this species. Social and demographic studies in the wild concur that breeding females live at least four times longer than their non-breeding members (S. Braude, pers. comm. 2013).

1.3 Systematics

NMR are mammals within the rodent order (Fig. 4). They are part of the suborder Hystricomorpha within which they belong to the Hystricognathi, parorder Phiomorpha. The latter includes the family Bathyergidae. The Bathyergidae encompass six genera and more than 22 species (Ingram et al. 2004; Kock et al. 2006; Deuve et al. 2008). Molecular phylogenetic analyses suggest that *H. glaber* is a sister lineage to the other subterranean bathyergids, e.g. *H. argenteocinereus*, *F. damarensis* and *F. darlingi* (Deuve et al. 2008). NMR are monotypic (Allen 1939) with a fixed chromosome number (60). So far, no morphological variation is known.

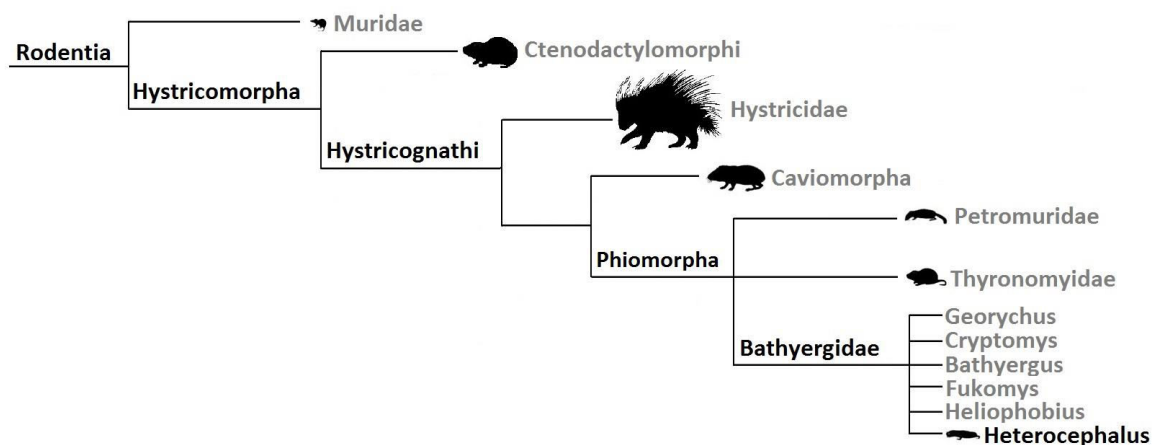


Fig. 4: The phylogenetic relationship of the rodent order (modified from Buffenstein 2005).

1.4 Distribution and habitat

NMR are restricted to arid and semi-arid and hot areas in the horn of East Africa (Kingdon 1970; Fig. 5). They are found throughout most of Somalia, central and east of Ethiopia and large parts of northern and eastern Kenya, almost extending as far south as to the eastern border of Kenya (Honeycutt et al. 1991a; Jarvis and Sherman 2002). NMR have also been seen and found near Ina-Assamo in Djibouti, a city located next to the border of Ethiopia and Somalia (Pearch et al. 2001). However, the species appears to have a wider distribution area than presently known.

Because of their subterranean habitat they are not easily to detect and many sights are not monitored regarding their presence.

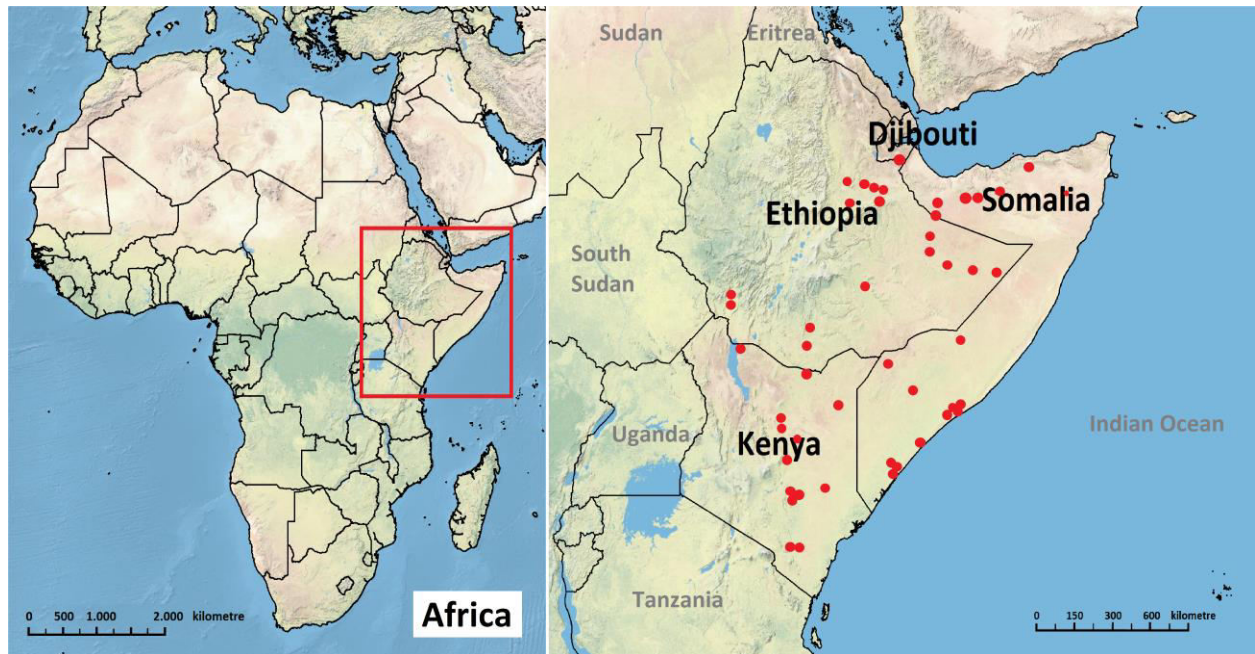


Fig. 5: Distribution of *Heterocephalus glaber* in the horn of Africa, presence of NMR represent by red dots.

The distribution areas are characterised by little diurnal or seasonal fluctuation, high ground temperatures, dry, sandy soils and unpredictable rainfall, which generally averages 200 to 400 mm/yr (Brett 1991a). Jarvis et al. (1994) showed that approximately 25 mm is the quantity of rainfall required to soften the earth at the depth of the burrows, which facilitates digging. In the wild, NMR live 0.5 to 2.5 m beneath under the earth's surface (Brett 1991a) in sealed, subterranean burrow labyrinths of up to a length of 2.3 to 2.9 km per colony (Brett 1991). During one year a colony of 85 to 90 NMR can achieve about 400 to 500 mole-rat hills; one colony is able to transport about 13.5 kg/hr of soil to the surface (Brett 1991a). Even when data on burrow spacing of NMR are limited, colonies appear to be usually located 400 to 1.000 m apart from their neighbours (Jarvis 1985; Brett 1986). Within the tunnel system, individuals dig burrows for different purposes, such as a nest-chamber, a chamber for food-storage and a waste chamber, as well as one to two toilets (Brett 1991). Life under the ground surface protects NMR from climatic extremes as well as from most pathogens and predators. This significantly decreases their levels of exogenous dangers and mortality (Buffenstein 1996).

NMR are not enumerated as an endangered or threatened species by the international Union of Conservation of Nature. However, given the patchy existence and distribution of this species (Brett 1991a) and their properties in term of population genetics (Allard and Honeycutt 1992; Bennett and Faulkes 2000), it cannot be ruled out that local, genetically distinct subpopulations are threatened. As NMR colonies are located in hot and dry areas with little farming and slow development, they are not a considerable agricultural pest. Nevertheless, extension of the human population into parts of NMR distribution areas will unavoidable results in conflicts. Conflicts arise particularly because NMR consume sweet potatoes and manioc, which are the main components of the local farming. NMR are protected in a number of Kenyan national parks and resorts, including Samburo, Kora, Tsavo and Meru, whereas their conservation status and currently distribution in Djibouti, Somalia and Ethiopia are still unknown.

Meanwhile procedures to conserve NMR in captivity have been developed and established (Jarvis 1991b; Artwohl et al. 2002; Sherman and Jarvis 2002). Zoological gardens and research institutes keep and bred NMR colonies in artificial burrows all over the world.

1.5 Adaptation

Fossil records uncovered that Bathyergidae ancestors have occupied a subterranean niche since the early Miocene era approximately 24 million yr ago (Maglio and Cooke 1979). Consequently, NMR display a number of anatomical specialisations for the life and survival in a subterranean environment (Fig. 6).

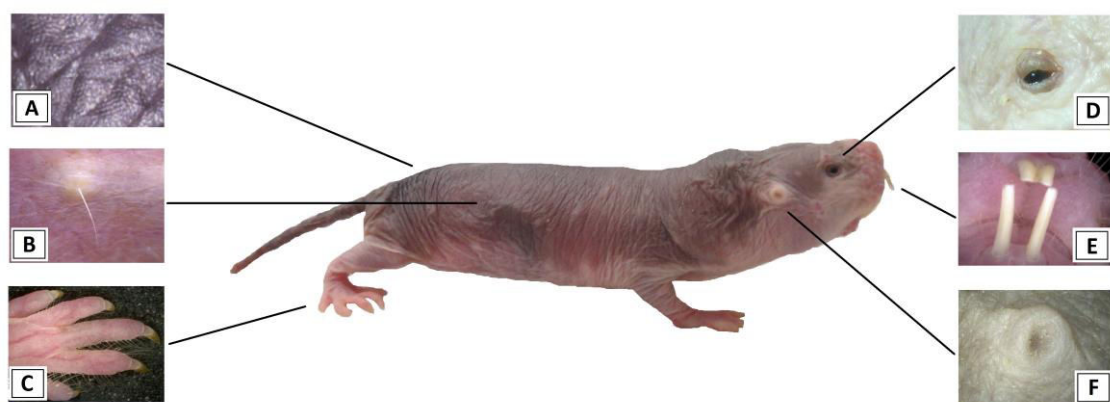


Fig. 6: Physical adaption of the NMR to subterranean life: A) Wrinkled, naked skin, B) Sensory hairs on the body surface for orientation in the dark tunnel system, C) Narrow feet with sensory hairs between the toes for location of burrow vibrations, D) Small eyes, E) Large, prominent and exterior located incisors for digging, F) Reduced, small ears.

NMR have an elongated body shape, with a kyphotic appearance of their dorsum. Their extremities are relatively slender and short (Fig. 6C). The head is small and spherical, blunt anteriorly, with a marginal rounded region at the back.

Particularly striking are the large and prominent incisors (Fig. 6E). They are permanently located exterior to the oral cavity and used for social interactions (Margulis et al. 1995; Schieffelin and Sherman 1995; Clarke and Faulkes 1997), digging (Catania and Remple 2002), object manipulation (Shuster and Sherman 1998), and feeding. Surprisingly, the mandibles connect at their rostral ends in a supple mandibular symphysis, allowing the lower incisors to move independently (Fig. 6E), thereby providing a greater range of teeth movements than typical for most mammalian (Catania and Remple 2002). In addition, one-third (31%) of the primary somatosensory cortex is attached to the representations of the lower and upper incisors, which impressively highlight the important role of the teeth for NMR (Catania and Remple 2002).

NMR are nearly hairless rodents (Fig. 6A), except for a sparse coverage with sinusoidal (sensory) hairs on the body surface (Fig. 6B). The occurrence of hair is limited to mechano-sensitive hairs scattered around the body, tail, face and hairs between the toes, similarly looking like fish bones (Fig. 6C). NMR depend on their sensory vibrissae on the body, which enable them to orient precisely towards sensory stimuli in dark and narrow burrows (Crish et al. 2003). The dorsal skin is shaded, by light pink and grey pigment cells, while the ventral skin is more sallow pink. The mixed skin colour of NMR emerge from a combination of the presence of blood vessels (Daly and Buffenstein 1998) and melanin. It may provide a kind of camouflage during the rare cases when adult NMR are above ground, such as e.g. dispersing animals or as “volcanoers”, which kick soil out of the burrow. The exemption to this coloured skin pattern include newborns, long established queens and breeding males as well as very old moles (Braude et al. 2001), all of which display an consistent pink body shape.

The eyes of NMR are significantly reduced in size (Fig. 6D). Most of the time they navigate and dig with their eyes closed in their deep burrows, implying that these subterranean rodents do not depend on vision. Nevertheless, this species have retained almost all of the well characterised cell types of the mammalian eye. However, the structural organisation of their eyes is considerably underdeveloped in comparison to diurnal mammals with better visual properties (Mills and Catania 2004; Nikitina et al. 2004). According to recent studies, NMR are able to detect amorphous light but no images (Hetling et al. 2005).

Regardless of their highly social structure and wide-ranging vocal repertoire of at least 17 different vocalisations (Pepper et al. 1991) over a narrow auditory range (65 Hz to 12.8 kHz at 60 dB; Heffner and Heffner 1993), their hearing notable degenerated. Like other subterranean species, NMR have no external pinna (Fig. 6F) and their capability to detect sounds is limited (Heffner and Heffner 1993). Reduction of the auditory and visual senses may have led to an enhanced detection of vibrations in this species (Quay 1981). Even if NMR do not drum (Bennett and Jarvis 1988a; Pepper et al. 1991) they are highly sensitive to ground vibrations (Jarvis 1978; Brett 1985).

Living in a nearly constant ambient temperature, NMR are unable to effectively regulate their body temperature. Despite the use of endogenous thermogenic features, as e.g. shivering, this species is thermo-conform to their environment (Buffenstein and Yahav 1991a; Hislop and Buffenstein 1994). Accustomed to a constant temperature in their burrows of nearly 30°C, their body temperature is generally only 1°C higher than the ambient temperature (Buffenstein and Yahav 1991a). NMR often rely on ectothermic mechanisms, such as huddling together in the nest, to maintain a constant body temperature (Woodley and Buffenstein 2002).

Captive NMR colonies are active around the clock and do not exhibit a circadian rhythm or a rhythm influenced by light cycles (Davis-Walton and Sherman 1994). However, Ricco and Goldman (2000) showed that it is possible to enable isolated individuals to exhibit circadian patterns of wheel-running activity. They can be accustomed to light and temperature cycles.

Physiological adaptations of NMR include low rates of gas exchange. In line with this purpose their haemoglobin shows a higher affinity for oxygen than that of most other mammals (Johansen et al. 1976) and their weight dependent metabolic rate is about one-third lower than compared to other subterranean or surf dwelling rodents (Buffenstein and Yahav 1991a). NMR show extreme resistance to oxygen deprivation (Larson and Park 2009). Apparently they lack the ability to sense chemical (capsaicin and acid) pain on the surface of their skin (Park et al. 2008). Additionally, in contrast to other species, morphine shows no analgesic effect on NMR (Kanui and Hole 1990). On the contrary, opiates induce fighting, extreme aggressive behavior and hyperalgesia as well as increased sensitivity to pain perception in this species (Towett et al. 2006; Busch-Dienstfertig et al. 2013).

1.6 Reproduction

NMR colonies exhibit a strong hierarchical order (Fig. 3); breeding is typically restricted to the “queen” and one to three males. These sexual relationships can remain stable for many years (Jarvis 1991; Buffenstein 2005). Breeding suppression in NMR of both sexes is not primarily facilitated by pheromones, but depends instead on physical aggression (shoving, biting; Faulkes and Abbott 1993; Clarke and Faulkes 1997; Smith et al. 1997) by the queen, leading to high levels of stress hormones in the addressed, non-breeding colony members (Faulkes and Abbott 1993). As a result, non-breeding females are anovulatory (Faulkes et al. 1990b). Also non-breeding males have lower plasma luteinizing hormone (LH) concentrations, urinary testosterone levels (Faulkes et al. 1991) and produce significant fewer mature spermatozoa than breeding males (Faulkes et al. 1994; Faulkes and Abbott 1997). Interestingly, all individuals of the colony, including males and even babies, pass through a magnification of the teats during the second half of the queen's gestation, even though only the breeding female lactates (Jarvis 1991; Jarvis and Bennett 1991). Considering the low sex steroid profiles in suppressed NMR, it is not astonishingly that these non-breeding workers are sexually almost monomorphic (Fig. 7).

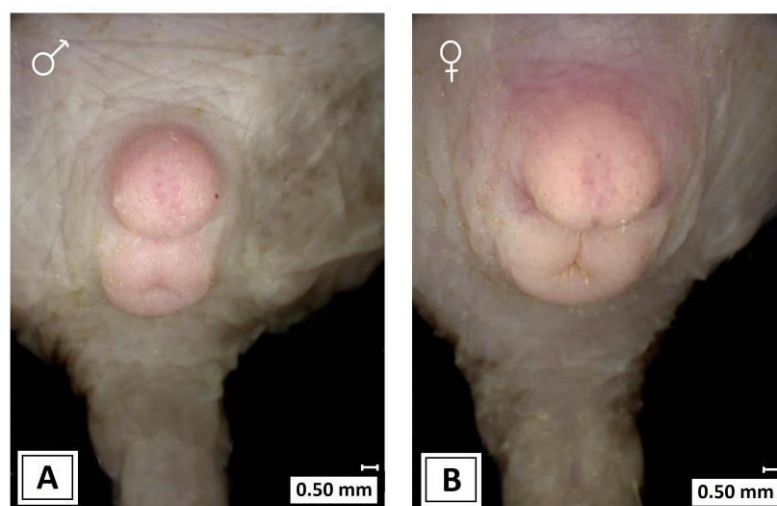


Fig. 7: Genitals of (A) male and (B) female naked mole-rats. The genital mound is anterior to the anal mound and appears similar in both sexes.

Both gender have equal behavioural patterns, body size, body weight, perineal muscles as well as motor neurons, spinal cord and brain structure (Jarvis 1991; Jarvis and Bennett 1991; Peroulakis et al. 2002; Goldman et al. 2006; Holmes et al. 2007; Holmes et al. 2008; Seney et al.

2009; Pinto et al. 2010; Edrey et al. 2011). Although the genitalia of NMR also appears to be almost equivalently in both sexes (Fig. 7), performing visually sex determination in NMR is a widely used method (Jarvis 1991; Braude 2000; Ludwig and Krönke 2012). This method is often controversial discussed in the literature as the visual determination of the sex inherits a high error rate.

Despite the sexual suppression, non-breeding NMR are becoming as soon as possible reproductively active when breeding opportunities arises. The possibility is provided if there is a relaxation of reproductive suppression, while the queen is dead, ill, injured, removed from the colony or just lost its power to shove colony mates effectively, such as when she is near parturition (Westlin et al. 1994). Compared to other subterranean mole-rats, an incest-avoidance mechanism do not seem to exist (Jarvis et al. 1994; Bennett et al. 1996) and new queens and most breeding males are recruit from the native family group (Braude 1991; Jarvis et al. 1994; Clarke and Faulkes 1997, 1998) resulting in low levels of genetic variation between the colony members and high levels of inbreeding (Faulkes et al. 1990; Reeve et al. 1990; Honeycutt et al. 1991b). As a matter of fact, less than 0.1% of all non-breeding females within a colony eventually get reproductive active (Jarvis et al. 1994; Buffenstein 2005). Leaderless colonies result in social instability followed by increased intracolony aggression and the possible death of some colony members of both genders during hierarchy fightings (Jarvis 1991; Lacey and Sherman 1991; Clarke and Faulkes 1997). Social order is restored when one female has taken over the new queen position on the top of the colony, and has selected her prospective breeding male(s).

Former studies showed that if social suppression signals are removed, non-breeding individuals go through internal and external changes, e.g. they gain body weight (Clarke and Faulkes 1997) exhibit elevated urinary testosterone titres (Clarke and Faulkes 1997), display increased aggressive and dominant behavior, and develop swollen genitals and active and enlarged gonads. Female workers exhibit a perforate vagina in less than seven days (Faulkes et al. 1990a) and increased progesterone levels in fewer as in 19 d (Faulkes et al. 1991a). In both sexes LH and urinary testosterone titres increase significantly (Faulkes and Abbott 1991).

Over the period of successful reproduction, the breeding pair undergoes further irreversible external changes. The queen experiencing a unique development; after multiple reproduction cycles her lumbar vertebrae restart to grow and further elongate (O'Riain et al. 2000; Henry et

al. 2007; Dengler-Crish and Catania 2009). The elongated body of the breeding female may facilitate her to accommodate large numbers of babies (the maximum recorded litter size counted 30 pups; R. Buffenstein, pers. comm. 2013) without increasing too much in body size. Otherwise she would not be able to defend her position as queen by patrolling the burrow system (Jarvis et al. 1991) to sexually suppress the other colony members. The queen significantly gains body weight, as many as 84% during gestation (Jarvis 1991). Furthermore, established queens are characterised by an enlarged and prominent mammae (Fig. 8B) and a significantly swollen vagina (Jarvis 1991; Henry et al. 2007) with an external, visible and succinct reddish line (Fig. 8A; Ludwig and Krönke 2012).

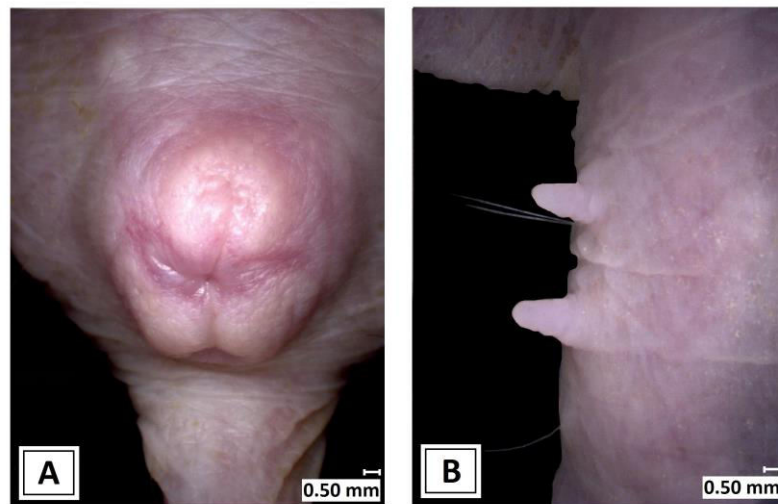


Fig. 8: Physical characteristics of a naked mole-rat queen: (A) swollen vagina with an external, visible and concise red line, (B) large and prominent teats.

Breeding males generally lose 17 to 30% of their body weight over their reign time, especially within colonies with high upbringing of offspring (Jarvis et al. 1991). Even though the process and function are still unknown, the rather greyish body color of the breeding pair gets brighter over the years into a light pink (Braude et al. 2001).

NMR are spontaneous ovulators, with a cycle length of approximately 27 to 28 d (Faulkes et al. 1991; Goldman et al. 2006), a post-partum estrus of 7 to 11 d (Jarvis 1991; Roellig et al. 2011) and a gestation period calculated to be 66 to 76 d (Jarvis 1991; Roellig et al. 2011) or 72 to 77 d (Lacey and Sherman 1991), respectively. Behavioral estrus lasts only 2 to 24 hr, during which repeated mating occurs (Jarvis and Bennett 1991), with a copulation time less than one minute

(Lacey and Sherman 1991). The observed interval between litters is quite variable in NMR colonies and can range from 77 to 84 d (Jarvis 1991; Brett 1991a) or 80 to 204 d (Roellig et al. 2011).

Reproduction of NMR is continuous throughout the year in captive as well as in wild colonies (Brett 1991a). Breeding individuals are capable of breeding over the entire span of their long lives (Buffenstein 2008). They may breed for more than 15 yr. The most productive, captive born breeding female lived for 23.5 yr and raised more than 900 babies in her 11-yr reign (Grimes et al. 2012). The oldest recorded breeding male was 28.3 yr old and successfully still sire a litter of newborns shortly before he died (Buffenstein 2005). In an established, regularly reproducing colony a breeding female can bear four to five litter per year (Jarvis 1991) with a mean litter size of nine pups for newly established and 16 pups for long-reigning queens (Buffenstein 2008).

Birthing can last several hours and is carried out by the queen without the help of the other colony members. Pups are nursed exclusively by the queen for about four to six weeks (Urison and Buffenstein 1995; O'Riain and Jarvis 1998; Dengler-Crish and Catania 2009; Roellig et al. 2011), depending on litter size and development level of the pups. Within a few hours after birth, the newborns already are able to walk (Roellig et al. 2011), about 14 d the auditory canal begins to open (O'Riain 1996), and after about 30 d the eyes are open (Jarvis 1991). About two to three weeks old pups attempt to eat little amount of solid food and begin to beg for feces from other colony members (Jarvis 1991; Urison and Buffenstein 1995; O'Riain and Jarvis 1998; Buffenstein 2005) and start to walk out of the nest (Stankowich and Sherman 2002).

2. Material and methods

2.1 Animals

The IZW holds the approval to keep and to breed NMR for research purpose only (#ZH 156; 23th of September 2008) by the “State office of health and social welfare” (Landesamt für Gesundheit und Soziales, Berlin).

The NMR colony I was founded at the IZW at the 19th of September 2008. The founding population consisted of 16 unrelated adult animals and included one queen (QI, that had been nulliparous before), five females and ten males, all of which were donated by the Rio Grande Zoo in Albuquerque, New Mexico, USA. Two individuals died within a week after the arrival.

After a breeding time of 11 mo and two successful litters of queen QI, the colony was divided into two different colonies. Queen QI was left in the original system with her two litters, the king and five adult males. Until now, this colony has produced 13 successful litters. The second colony was founded with the remaining adult NMR, namely two females and nine males from the founding population. After two months, one of the females became the new queen of colony II. Though queen QII held her position in the new colony, she did not produce live offspring in the following months, supposedly because of a persistent uterus infection (Roellig et al. 2011). To achieve reproduction success, five more NMR (three females and two males from colony I) were transferred to colony II, eight months after the separation of the two colonies. Consequently, fights between the individuals developed, resulting in the death of queen QII as well as of three adult males. Two months after the death of the old queen QII, another female established herself as the new queen QII*. Queen QII* produced the first four successful litters in colony II. However, queen QII* was also killed by another female one year later. After two months of fighting, the latter became accepted as the new queen QII**. In contrast to the other queens of colony II, the third queen successfully claimed her position and produced six litters until today.

Overall, in the two colonies during four years, the three queens (QI, QII* and QII**) had 23 successfully pregnancies (L1-L23). At present the IZW accommodates 94 animals, excluding 12 NMR that were delivered to different zoos in Europe (Vienna, Budapest).

2.2 Housing and husbandry procedures

The colonies are kept at the IZW in an artificial burrow system (Fig. 9).



Fig. 9: Artificial burrow system for the NMR at the IZW.

The burrow system consists of eight boxes (plexiglass) which are connected by plastic tunnels (Fig. 9). The total tunnel length approximates 5.70 m. The boxes are defined by the animals themselves as nest chamber, toilet or food store-room. The chambers contain wood shavings for bedding, unbleached paper tissue as nesting material and small pieces of wood as enrichment and for scuffing their teeth. The animals were housed under semi-natural conditions, i.e. conditions that mimic their subterranean burrows in the wild. To achieve this, the temperature is modulated between 24 to 28°C (Brett 1986, 1991) via a heating coil positioned around the burrow system. The high relative humidity of 40 to 70% that is present in their burrow system under natural conditions (McNab 1966; Withers and Jarvis 1980) is achieved by fixing a wet paper tissue below the lid inside of each of the boxes as well as by placing several water-filled containers outside of the system. Furthermore, to sustain this customised environment for the NMR colonies, the whole tunnel system is enclosed by a large,

individual designed thermo box (engineering office Frank Butter; 2 x 1 m²; Fig. 9). In analogy to their subterranean burrows in the wild, the NMR in the IZW were kept in darkness, with the exception of one to two hours every morning for the daily maintenance of the system and handling of the animals. The toilet boxes were cleaned on a daily basis in the afternoon, the other chambers depending on colony size and accumulated waste.

Because NMR are highly sensitive to vibration and noise (Jarvis 1978; Brett 1985), a radio was used (8:00 am to 8:00 pm) in the NMR room to desensitise the individuals and to accustom them to noise and human presence.

Fresh food was given every day at 1:00 pm and included vegetables and fruits, e.g. sweet potatoes, carrots and other roots and tubers, as well as apples and grapes. In addition, a supplement of rat pellets, containing cereal and proteins enriched with vitamins and minerals was provided three times per week.

2.3 Animal identification

Due to the fact that both sexes of NMR can hardly be visually distinguished, they were individually tagged according to a numerical pattern for identification. Within 12 hr after birth, newborns were marked by toe-clipping using surgical scissors. This method has been successfully performed previously (Brett 1991; Braude and Ciszek 1998) and was established at the IZW after the second successful litter of queen QI.

Because of the limits of this method, all pups further receive a transponder microchip (HT 157; GuangZhou hongTeng Barcode Technology Co., Ltd., Guangzhou, China) about three months after birth. This is also an established method in many other NMR colonies (Braude and Ciszek 1998; Goldman et al. 2006). For the latter procedure NMR are captured and placed in a large inhalation anesthesia facemask. General anesthesia is induced using an administration of initially 6 Vol. % isoflurane (Isoba, Essex) oxygen flow rate of 1 l/min using a Dräger Vapor 2000 system. Once the animal is asleep, inhalation anesthesia is maintained using a smaller mask suitable for rodents at (1.5 to 2.0 Vol. % isoflurane). To prevent, hypothermia all procedures (anesthesia, ultrasound and inserting transponder microchips) are performed on an electric heating pad (HT 200, Minitub GmbH, Tiefenbach) operating with 37°C. Furthermore, the health

status of the NMR are briefly checked by Prof. Hildebrandt (Hildebrandt et al. 1998), using high-frequency, high-resolution ultrasound (Vevo 2100, VisualSonics Inc., Toronto).

After performing ultrasound, NMR are positioned on their abdomen and the transponder-chip (size: 7 x 1 mm) is injected subcutaneously with a customer-built injection needle on the back side of the left shoulder. To facilitate healing, the edges of the resulting small wound are adapted by an adhesive spray (Leukoplast, BSNmedical).

2.4 Weighing

To control health condition of the NMR, they were weighed within regular time intervals.

Starting on their day of birth, surviving newborns were weighed on a daily basis at about 10:00 am over the first 80 d postpartum. This period corresponds to the average inter-litter interval, and was the same interval used by Bennett et al. (1991) and O`Riain (1996) for NMR and other bathyergidae species. The newborns were collected and removed from the nest, identified, based on their individual toe-clipping pattern and temporarily accommodated on a heating pad until they were weighed. After reaching an age of 80 d, weighing was performed only twice a month together with the rest of the NMR colony. The older NMR were identified by their transponder-chips. Body weights were measured using a digital top-loading balance (Kern 822-37, Gottl. Kern & Sohn, Albstadt).

2.5 Molecular sexing

Due to the fact that NMR pups exhibit no sex differences in external genitalia, the sex ratio of the offspring was determined via a molecular sexing method, the polymerase chain reaction (PCR; Fig. 10).

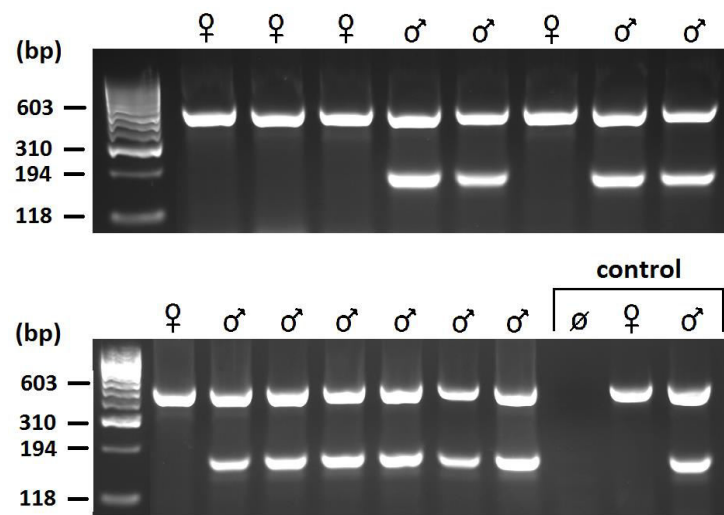


Fig. 10: Molecular sexing pattern of the 12th litter of queen QI (5♀ + 10♂). ♀: female, ♂: male, ∅: distilled water. Molecular sizes of the band are indicated in base-pairs (bp) on the left lane. Hyperladder IV was used as a molecular size marker.

The cut-off-toe sample obtained in the context with the identification of the newborns was used as a DNA source. The cut-off-toe of each pup was collected in a 1.5 ml Eppendorf tube and subsequently snapped frozen in liquid nitrogen (-196°C). A modified PCR protocol for molecular sexing of NMR developed by Katsushima et al. (2010) was used. This multiplex PCR amplification method includes specific primer sets of the DBY gene and the mitochondrial 16S rDNA. These primers generate two DNA bands, a male-specific band of the Y-chromosome-linked DBY gene (163 bp) in males and a common band of 16S rDNA (446 bp) in both, males and females. As shown in Fig. 10 this PCR method produce two DNA bands (163 bp + 446 bp) in all males, and accordingly one band, the common band (446 bp), in all females.

The complete sex ratio at parturition was determined for L3-L23. Due to the fact that the first queen QI was already pregnant at arrival and the sexing methods had to be established at the IZW, no genetic samples of her first two litters (L1 and L2) had been taken and thus the sex ratio of these two litters were not analysed.

2.6 Photo documentation

The photos used in this thesis were either taken using a Keyence microscope (VHX-550 microscope, VH-Z20R 20-200x zoom lense, Keyence Corporation, Japan), or a digital camera (Canon IXUS 105).

2.7 Statistics

All statistical analyses were performed using SPSS version 18.0.0. (SPSS, Chicago, IL, USA). The Shapiro-Wilk-test was used to test data for normal distribution. Normally distributed data of two conditions were analysed using the Student`s t-test, for data involving more variables, a simple analysis of variance (ANOVA) was performed. Otherwise, non-parametric tests were applied. For two independent samples, the Mann-Whitney U-tests was applied and for more than two variables the Kruskal-Wallis H-tests. In case of normally distributed data correlation statistics were carried out using the Pearson correlation; otherwise Spearman rank correlations were applied. Significant values were obtained from two-tailed statistics. Sample sizes differ within the data sets as reliable information could not be obtained from each focal individual for all variables e.g. weight, survival rate.

3. Results

Reproductive success

On 19th of September 2008, the IZW started breeding NMR with a small population of 14 individuals (six females and eight males). The initial colony was split after 11 mo. In the last four yr, these two NMR colonies developed into very successfully breeding colonies, with each queen rearing up to four litters per year (Tab. 1). Until now, a total of 23 litters with 273 pups have been born by three different queens (QI, QII*, QII**).

The first colony with its queen QI (body weight: ~47 g, length: 17 cm) gave birth to 13 litters (L1-L13) with a total number of 153 pups until today (Tab. 1). In the second colony, founded on 21st of August 2009, two subsequent queens successfully reproduced four litters (L14-L17) and 50 pups were born by queen QII* (body weight: ~55 g, length: 17.5 cm) and six litters (L18 to L23) and 70 pups by the present queen QII** (body weight: ~58 g, 19.5 cm; Tab. 1).

During this study one mating in colony I on the 16th day after the last birth of the previous litter was observed.

Tab. 1. Litter data (size and sex) of the three NMR queens, for the years 2008-2012

		L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13		
Queen QI	Litter	1	2	3	4	5	6	7	8	9	10	11	12	13		
	LS	11	12	11	14	9	6	9	16	14	18	10	15	8		
	♂/♀	-	-	4,7	8,6	7,2	4,2	6,3	8,8	9,5	9,9	4,6	11	4,4	Σ 153 pups	
		L14	L15	L16	L17											
Queen QII*	Litter	1	2	3	4											
	LS	13	11	11	15											
	♂/♀	5,8	5,6	4,7	8,7											Σ 50 pups
		L18	L19	L20	L21	L22	L23									
Queen QII**	Litter	1	2	3	4	5	6									
	LS	12	8	20	15	10	5									
	♂/♀	8,4	6,2	11,9	11,4	6,4	-									Σ 70 pups

QI= queen of colony I; QII*= second queen of colony II; QII**= third queen of colony II; LS= Litter size; ♂=Male, ♀=Female.

Inter-birth intervals

Surprisingly, the first litter (L1) was born just 48 d after the founder population arrived at the IZW. Considering the gestation period of ~70 d (Jarvis 1991; Lacey and Sherman 1991; Roellig et al. 2011), queen QI must have been pregnant prior to her arrival.

The mean inter-birth interval of the colonies was 103.55 d ($n=20$, $SD=39.37$), ranging from 72 to 203 d. Compared to the two other queens, QII* exhibited the shortest mean birth interval with 79.67 d ($n=3$, $SD=0.58$). With a mean interval of 117.17 d ($n=12$, $SD=45.46$), queen QI showed the longest and most variable inter-birth interval (80 to 203 d) of all three queens. There was no significant difference between the birth intervals after successfully ($\geq 50\%$ of the pups survived) raised litter in comparison to birth intervals after unsuccessful (survival of $\leq 50\%$ of the pups) one (Mann-Whitney U-Test, $n=20$, $U=48$, $Z=-0.11$, $p=0.91$).

Litter size

The average litter size in the colonies was 11.87 ($n=23$, $SD=3.68$; L1-L23) and ranged from 5 to 20 pups (Tab. 1). There was no significant difference in litter size of the three queens (Kruskal-Wallis Test, $n=22$, $\chi^2=0.38$, $df=2$, $p=0.83$; L1-L22). Also, the number of previous pregnancies of a queen did not significantly affect the size of the subsequent litters (Spearman rank correlation, $n=22$, $r=-0.01$, $p=0.98$). Increasing litter size led to a significant reduction of the birth weight of newborns as shown in Fig. 11 (Spearman rank correlation, $n=242$, $r=-0.37$, $p=0.00$).

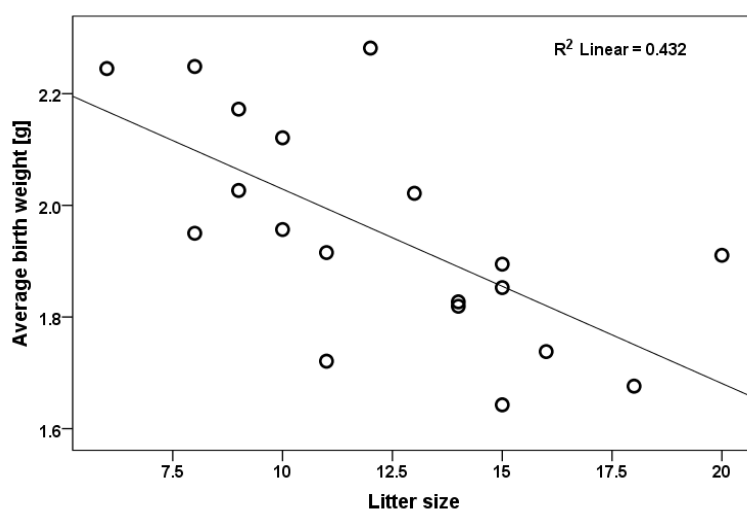


Fig. 11: Relationship between total litter size and mean birth weight of the single litters of captive born naked mole-rats at the IZW. Each dot represents one litter.

Birth weight of newborns

The mean birth weight of all neonates in the colonies was 1.90 g (n=243, SD=0.20; L3-L22) and ranged from 1.05 g to 2.65 g (Fig. 12).

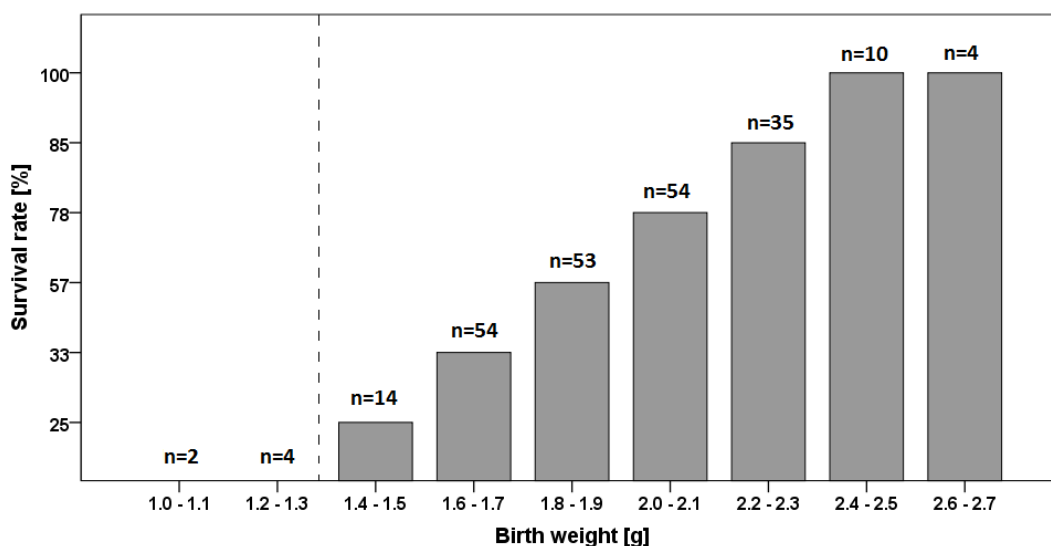


Fig. 12: Survival rate of newborn pups according to their birth weight.

Offspring with a weight of less than 1.35 g at birth (n=6), did not survive in this study (Fig. 12). There was a trend for heavy born pups to have a higher probability of survival. Newborns with a rather low birth weight showed also a low survival rate. For example, individuals with a body weight of ≤ 1.75 g only survived in 33% of the cases, whereas pups born with a birth weight of ≥ 2.20 g had a survival rate of 85%. Of the 14 pups with a birth weight ≥ 2.4 g, all survived the first three months. In addition, the mean birth weight of the offspring surviving for at least three months was 2.05 g (n=107, SD=0.25), which is significantly higher than that of pups which died (1.77 g; n=134, SD=0.27) during this period (t-Test, n=241, F=0.18, df=239, p=0.00).

There was also a significant difference between the birth weights of newborns of the different queens (Kruskal-Wallis Test, n=232, $\chi^2=24.66$, df=2, p=0.00). The average birth weight of the newborns of queen QII** was 2.04 g (n=63, SD=0.34), which is significantly heavier than that of the pups of queen QI with 1.84 g (Mann-Whitney U-Test, n=185, U=2168.50, Z=-4.85, p=0.00) and 1.87 g of the pups of queen QII* (Mann-Whitney U-Test, n=110, U=940.50, Z=-3.26, p=0.00). The birth weights of the neonates of queen QI (n=122, SD=0.24) and QII* (n=47, SD=0.39) did not significantly differ (t-Test, n=169, t=-0.81, p=0.42).

The female newborns, with an average birth weight of 1.85 g ($n=107$, $SD=0.29$), weighed significantly less than the male ones with 1.93 g ($n=136$, $SD=0.29$; t-Test, $n=243$, $F=0.66$, $df=241$, $p=0.02$). Pups with a birth weight below 1.90 g had a significant higher risk to die in the first three month period than pups with a birth weight above the average birth weight (Mann-Whitney U-Test, $n=123$, $U=719.50$, $Z=-4.76$, $p=0.00$).

Growth of the pups

The average growth curves based on body weight for female and male NMR pups of 20 litters (L3-L22; three different queens) are presented in Fig. 13 for the first 80 d postpartum.

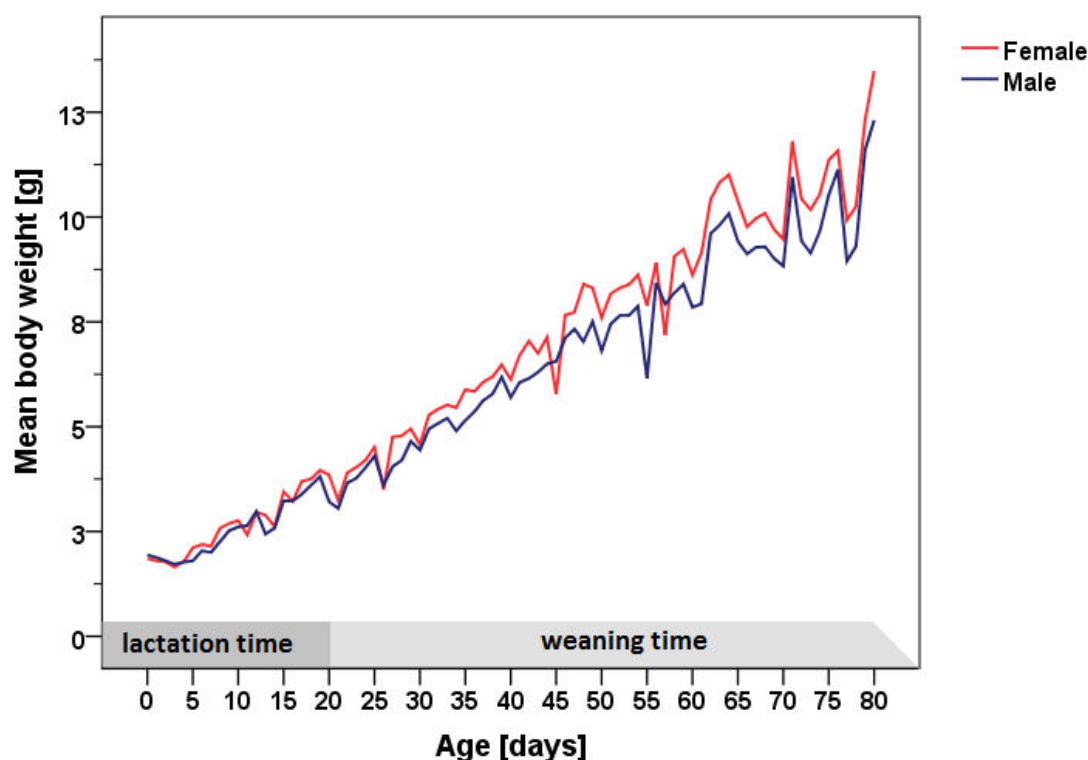


Fig. 13: Postnatal mean body weight for female and male NMR pups of twenty litters (L3-L22) of all three queens plotted against age.

This time period was chosen to examine the mean body weights of (i) newly suckled NMR pups and (ii) their variation during the weaning period. For the first 20 d NMR pups exclusively suckle (lactation time). After lactation time pups also start to eat solid food (weaning time), even

though the queen lactates them up to an age of 80 d. In exceptional cases the weaning time may be prolonged, for example by the death of the following litter or the occurrence of development-delayed pups.

Because of the high mortality rate of pups during the first postnatal weeks, many neonates did not survive the study interval, thus no data for these pups was available up to an age of 80 d.

In general, newborns lost body weight in the first days after birth and then started to gain body weight over the next few weeks (Fig. 13). The neonates of this study were born with an average body weight of 1.90 g ($n=243$, $SD=0.20$; L3-L22) and within 80 d achieved a mean body weight of 14.12 g ($n=7$, $SD=1.88$; L3-L22). Both sexes exhibited a continuous growth during the first postnatal weeks, with high inter-day variations. The sex of a pup seems to slightly influence the growth. As presented in this study, female NMR neonates were born with lower birth weight than their brothers, but on average, they gained body weight faster over the first 80 d postpartum than male ones. However, this more rapid weight gain of female pups seems to level out above an age of 80 d (Fig. 14).

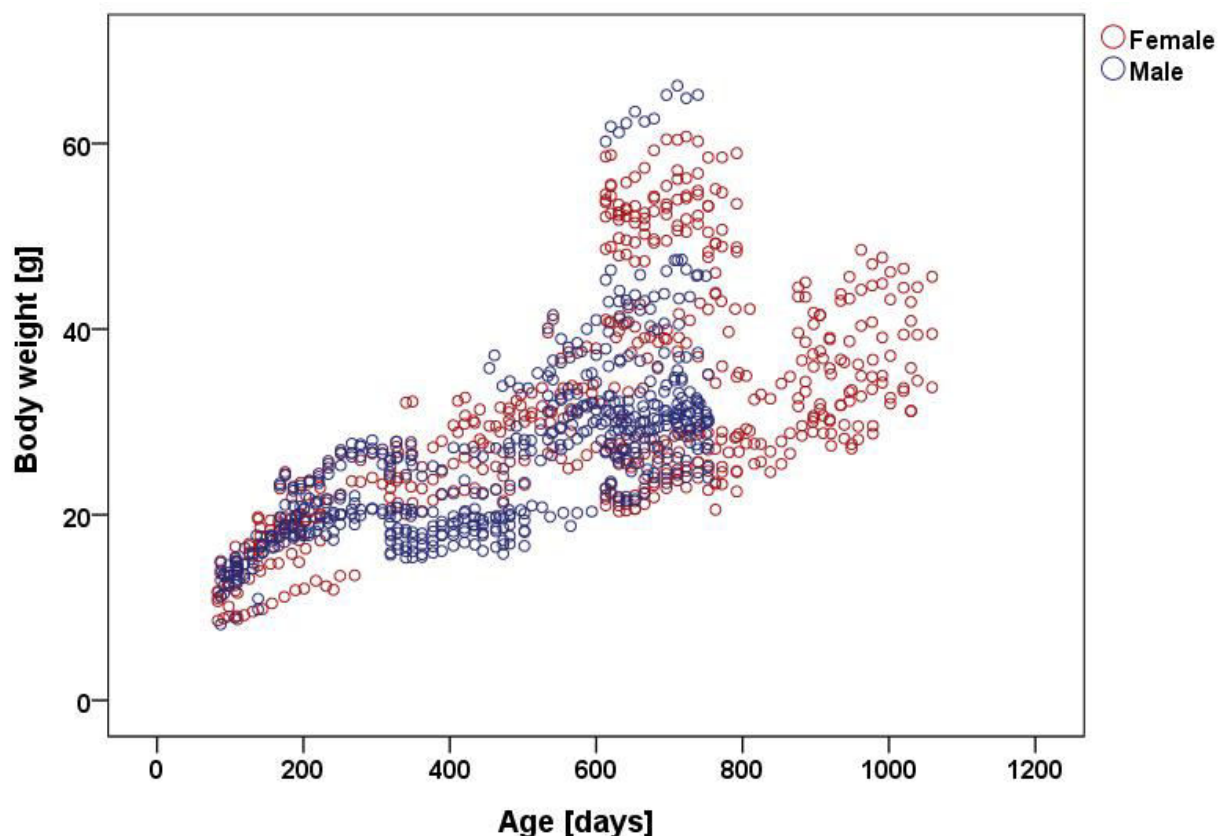


Fig. 14: Growth curve of NMR above an age of 80 d.

The change in body weight for all NMR of the two captive colonies ($n=99$) above an age of 80 d is presented in Fig. 14. Body weights of adult NMR seem to be gender-independent above an age of 80 d.

In this study, pups exhibit a high variation in body weight within and between single litters. As graphically shown in Fig. 14, an extremely rapid increased in body weight occurred in pups of the first and second litter (L1, L2; L14, L15) of both colonies at an age of ~ 700 d. For example, the nine 696 d-old pups of the first litter of QII* (L14, $n=9$) were significantly heavier than the ones born in later litters, e.g. litters with 794 d-old ones (L5, $n=6$; Mann-Whitney U-Test, $n=15$, $U=2.00$, $Z=-2.95$, $p=0.00$). Most of these NMR also tend to become the heaviest individuals in the whole colony.

There is also wide scattering of body weight within a litter between the single littermates. The body weight of the first litter of QII* ($n=7$; 696 d-old) ranged from 38.39 to 60.44 g. This phenomenon was almost evident in every litter, regardless of its age. At birth, newborns within the same litter had nearly a similar body weight (1.05 to 2.65 g) in this study. Successively, the wide scatter in body weights seems to increase over the first 80 d postpartum and maintained the whole study period.

The NMR in a colony seem to develop with different growth rates, particularly above an age of three months. Thus, there was no correlation between body weight and age of NMR pups over 80 d of life up to an age of 1100 d (Fig. 15).

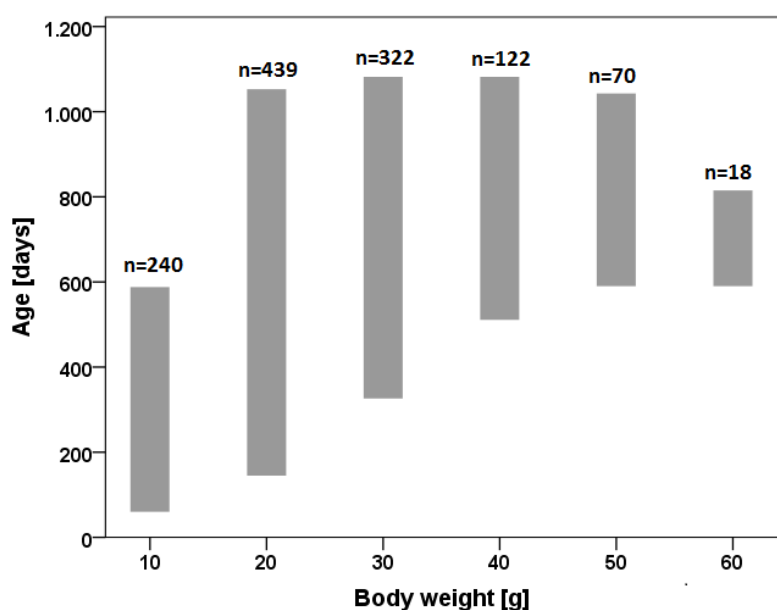


Fig. 15: The range of body weights in NMR according to their age above 80 d.

Above an age of three months, the body weights of the captive NMR ranged from 10 g to 60 g (Fig. 15). As shown in this figure, especially middle aged animals (>800 d) exhibited a wide range of body weight (20 g-50 g). Because of the variation in body weights, NMR pups may take 5-38 mo to attain the mean body weight of captive NMR (~30 g). But a few individuals of each colony may never attain this mean body weight. For example, at a body weight of 20 g, four animals more than 1030 d-old weighed less than 30 g.

In this study, the oldest animals (1059 d) were also not the heaviest ones (Fig. 15). This demonstrates that a comparatively small body weight (~30 g) can also occur in old NMR. The considerable variation in body weights in NMR shows, that the body weight of these animals is extremely plastic and not necessary related linearly to age during this study.

In this study there were also ten unsuccessful reared litters ($\leq 50\%$ of the pups survived). One of these litters was the 12th litter (L12, n=15) of queen QI, in which all newborns died within the first two weeks (Fig. 16A).

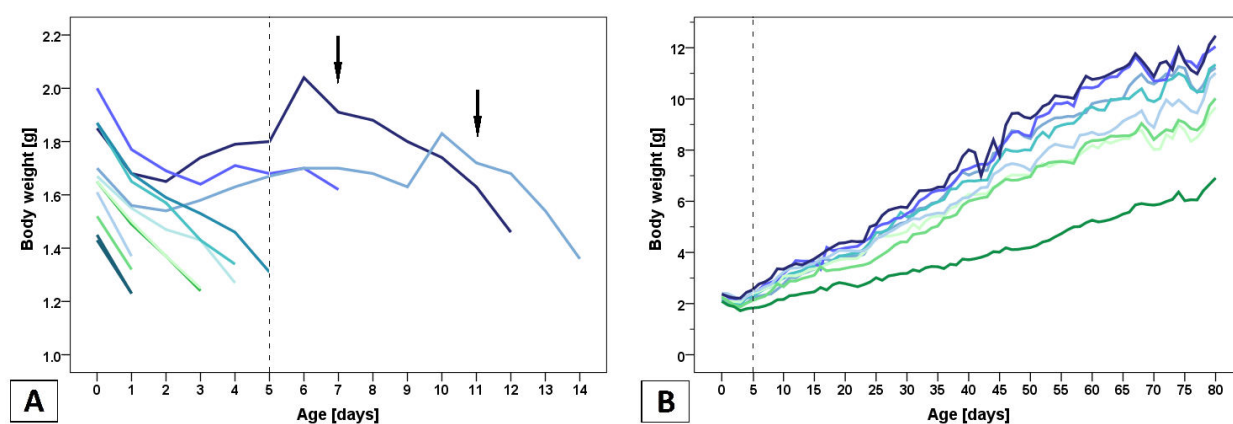


Fig. 16: **A)** Growth curve of a unsuccessful reared litter, the 12th litter (L12; n=15 individuals) of queen QI (during the extensive renovation work at the IZW). Each individual pup is depicted in a different color. The arrows depict the days with most ground vibrations at the institute caused by asphaltting the parking area next to the NMR housings, **B)** Growth curve of a successfully reared litter over the first 80 d postpartum, the 6th litter of queen QII** (L22; n=8 individuals).

This 12th litter of QI was born during extensive renovation works at the institute (IZW) next to the NMR housings. Similar to successful reared litters (Fig. 16B) the pups of unsuccessful litters also lost body weight over the first five days after birth. However, in contrast to successful reared litters, in unsuccessful litters more than 50% of the pups died within the first days. The

growth curves of the surviving pups in the first days after birth were characterised by extreme fluctuations and lastly a strong decrease of the body weight until they died (Fig. 16A).

Three of the pups with higher birth weights of this litter survived for the first five days and continued growing until day six. In the course of the daily renovation work, the parking area next to NMR housing was asphalted at day seven and eleven (Fig. 16A, the arrows depict asphaltting work), accompanied with heavy ground vibrations. During the next day the three pups lost in body weight, at the end of the day one further pup died. After the second day with strong ground percussions (day 11), both surviving pups continuously lost body weight over the next day(s) and finally died.

Sex ratio of the pups

Sex ratio of the complete litter at parturition has been determined for 245 pups (L3-L22; Tab. 2). This was due to the fact that solely in these litters genetic samples for sexing could be taken from all newborns.

Tab. 2. Data related to reproduction of the three breeding queens among different time periods

ID	Litter	Birth date	interval	Birth		Birth		5 days		1 month		3 months		1 year		2 years	
				mean weight (g)	LS	SR %	MR %	SR %	MR %	SR %	MR %	SR %	MR %	SR %	MR %	SR %	MR %
QI	1	06.11.2008	192		11						64		73				
	2	17.05.2009	203		12						33		42				
	3	06.12.2009	81	1.72 ± 0.16	11	36	0	25	27	25	27	25	27	25	27	29	36
	4	25.02.2010	88	1.82 ± 0.17	14	57	0	54	7	44	36	20	64	20	64	25	71
	5	24.05.2010	80	2.03 ± 0.27	9	78	0	86	22	86	22	86	22	100	33	100	33
	6	12.08.2010	146	2.24 ± 0.22	6	67	0	67	0	67	0	50	33	50	33	50	33
	7	05.01.2011	80	2.17 ± 0.20	9	67	0	67	33	50	55	67	67	67	67		
	8	26.03.2011	82	1.74 ± 0.21	16	50	13	50	75	50	75	33	81	33	81		
	9	16.06.2011	137	1.83 ± 1.73	14	64	0	58	14	64	21	70	28	70	29		
	10	31.10.2011	142	1.68 ± 0.20	18	50	0	57	61	57	61	57	61	57	61		
	11	21.03.2012	86	1.96 ± 0.21	10	40	10	33	10	30	40	20	50				
	12	15.06.2012	89	1.64 ± 0.23	15	67	7	75	73		100		100				
	13	12.09.2012		1.95 ± 0.28	8	50	13	0	88		100		100				
QII*	14	30.08.2010	79	2.02 ± 0.20	13	38	0	36	15	30	23	30	23	30	23	77	23
	15	17.11.2010	80	1.92 ± 0.31	11	45	0	50	9	71	36	71	36	71	36	64	36
	16	05.02.2011	80	1.74 ± 0.35	11	36	0	40	54	40	54	100	91	100	91		
	17	26.04.2011		1.85 ± 0.16	15	53	0	36	27	0	87		100		100		
QII**	18	27.07.2011	114	2.28 ± 0.21	12	67	0	64	8	64	8	60	17	60	17		
	19	18.11.2011	77	2.25 ± 0.15	8	75	0	71	12	71	12	71	12	71	12		
	20	03.02.2012	81	1.91 ± 0.18	20	55	0	67	40	60	50	60	50				
	21	24.04.2012	82	1.89 ± 0.35	15	73	0	83	60	100	93	100	93				
	22	15.07.2012	72	2.12 ± 0.33	10	60	0	50	20	50	20	50	20				
	23	25.09.2012			5		100		100		100		100				
Mean			103.55	1.90	11.87	56	7	53	36	53	49	57	56	58	48	57	39
SD			39.37	0.20	3.68	13.21	21.81	20.91	29.73	23.48	31.57	25.47	31.10	25.98	28.70	28.82	16.55
Total		23			273												

ID of the respective queen; *interval* = the time between two litters; *LS* = litter size; *SR* = sex ratio for males; *MR* = mortality rate;

As shown in Fig. 17, the sex ratios of all litters (of the three queens) were male skewed during the study period (2010–2012).

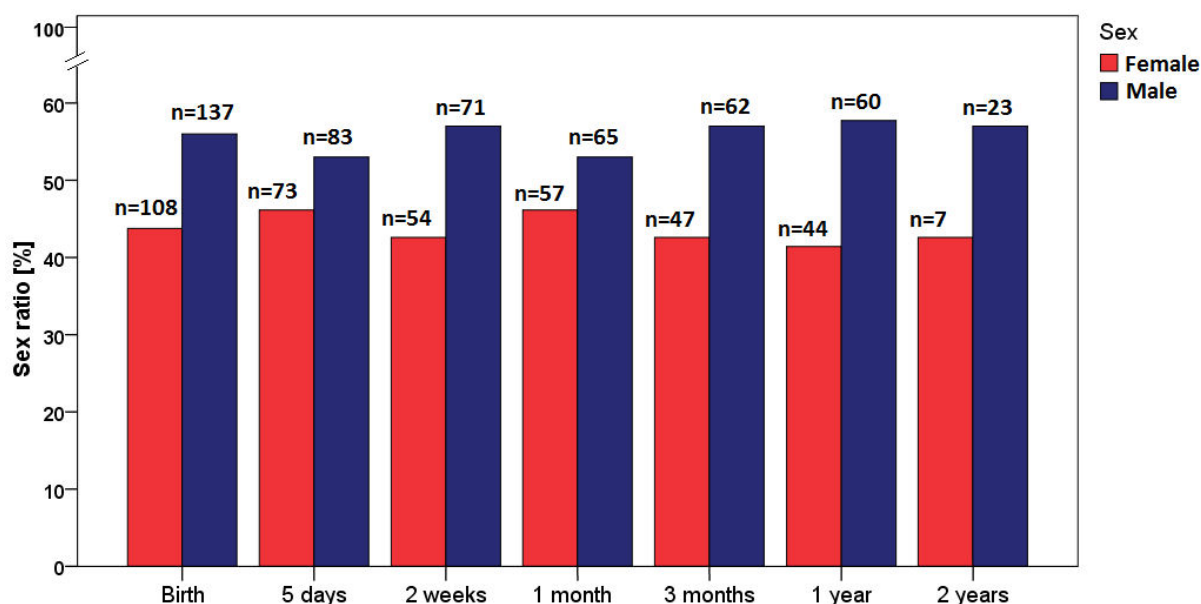


Fig. 17: Sex ratio of the litters L3-L22 to different age periods.

At birth, the sex ratio within the litters was slightly skewed to males with an average of 56% ($n=245$; L3-L22; Tab. 2; Fig. 17). As the mortality in NMR pups is highest during the first days of life, sex ratio oscillated during the time after birth. After 5 d the average sex ratio of all litters became less shifted towards males, decreasing to 53% ($n=158$; L3-L22). After two weeks, the male skewed sex ratio increased to 57% ($n=123$; L3-L22). Most of the pups that reached an age of two weeks were also still alive after one month, thus the sex ratio remained slightly male moved to 53%. Also, after three months, the mean sex ratio of all litters continued to be male skewed at 57% ($n=106$). For the litters in which data were available up to an age of one ($n=81$) or two years ($n=41$), respectively, the average sex ratio in the colonies continued to be slightly male shifted at 53% to 58%.

However, the three queens differed in the sex ratio of their single litters during different age classes of their pups. All litters of queen QII** (L18-L23) were considerable male skewed at birth and this sex ratio remained unchanged up to a pup age of two years (Tab. 2). In contrast to queen QII**, two of the thirteen litters of queen QI (L3 and L11) were female skewed at birth. These two litters remained female biased until the whole study period. In addition, two

more litters (L4 and L8) of queen QI were male biased at birth, but showed a female skewed distribution at a pup age of 1-3 mo. Interestingly, almost all litters of queen QII* were obviously female biased at birth (L14-L18; Tab. 2). This female skewed sex ratio remained unchanged until the pups reached an age of three months, afterwards the sex ratio of these litters was equally distributed between males and females (n=18; L14-L16). However, although the three queens exhibited variations in their percentage of male and female pups during the weaning period, in general, they showed no significant difference in their litter sex ratio (Fisher's Exact Test, $p=0.21$).

Mortality of the pups

The colonies exhibit high mortality rates for pups (Tab. 2; Fig. 16A; Fig.18). More than 58% of the 273 neonates died within their first postnatal weeks.

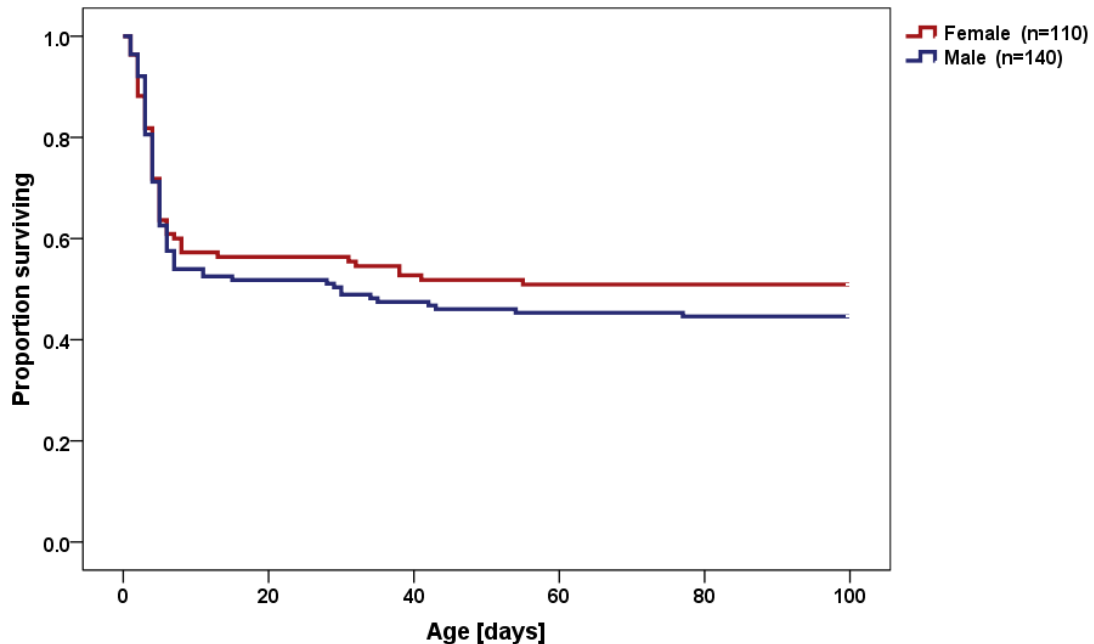


Fig. 18: Kaplan-Meier graph with survival curves of the captive born female and male NMR pups from birth to an age of 100 d.

On their day of birth, the pups of the three queens did not significantly differ in survival rate (Fisher's Exact Test, $p=0.17$).

On average, 64% ($n=21$, $SD=0.30$; L3-L23) of the newborns survived until the fifth day after birth, a time period during which most of the surviving pups also regain their birth weight. After one month (~start of weaning; Fig. 13) 51% ($n=23$, $SD=0.31$; L1-L22) of the pups were still alive, and only 44% ($n=23$, $SD=0.31$; L1-L23) reached their third month of life. At an age of three months, NMR pups are completely weaned and independent. After the weaning period, mortality rate is pretty low and 99% of the pups reaching the age of three months survived the study. Interestingly, for the first time the survival of the offspring significantly differed between queens from an pup age of two years on (Fisher's Exact Test, $p=0.05$).

Queen QII** was most successful, rearing 51% ($n=6$, $SD=0.39$; L18-L23) of her pups. The two other queens were less successful, as only 42% ($n=13$, $SD=0.27$) of the pups of queen QI and 37% ($n=4$, $SD=0.38$) of queen QII* survived to an age of two years.

The whereabouts of 131 dead pups were documented. Overall, 54% of the dead offspring disappeared and could not be retrieved anymore. They most likely were consumed by other colony members. The remaining 46% dead pups were either found in the toilet chamber or in the nest. The most frequent cause of pup death seemed to be undernourishment, but many of them also exhibited traces of cannibalism.

There was no significant difference between gender of the pups and time of death (Mann-Whitney U-Test, $n=124$, $U=1638.00$, $Z=-1.09$, $p=0.28$).

There was no significant correlation between survival rate until the age of three months and litter size in this study (Pearson Correlation, $n=23$, $r=0.15$, $p=0.51$). However, a significant positive correlation was found between colony size and the mortality rate of pups at an age of three months (Pearson Correlation, $n=23$, $r=0.42$, $p=0.05$).

The mortality rate at an age of three months of litters born between 03.02.12 and 25.09.12 (= time period of renovation work) was significantly higher than that of litters between the 06.11.08 and 31.10.11 (Fisher's Exact Test, $p=0.05$). At the end of the renovation work, queen QII** gave also birth to her last litter (L23) in this study. This litter consisted of very few newborns (five pups) that were all born dead.

With advancing age of queen QI the mortality rate of her litters also significantly increased (Pearson Correlation, $n=23$, $r=0.41$, $p=0.05$), which however, was due to the occurrence of an extremely high mortality rate of offspring at the end of the study period.

4. Discussion

The following paragraph is intended to discuss the results of this thesis based on the existing literature. Many aspects of pup ontogeny and reproduction of NMR were already discussed in the literature, but not in the same extent as in this thesis. This study is characterized by a huge sample size ($n=273$) of NMR pups, long-established breeding colonies, high survival rate of pups and a long-term study period of four years. Thus, the collected data may present an important contribution and comparison to other mole-rat species.

Inter birth interval

Breeding in NMR colonies occurs throughout the entire year in captive as well as in wild colonies (Jarvis and Bennett 1991). Queens may breed continuously (approximately every three months) from the time they established oneself as breeders until they die (Buffenstein 2008).

Inter-birth intervals in the colonies of the IZW were found to be considerably variable (72 to 203 d, $n=20$) with a mean inter-birth interval of 103.55 d (Tab. 2). Bibliographic references of other captive colonies prove that the same holds true for other breeding colonies. In the colonies of Jarvis (1991), the breeding females gave birth in very short intervals of on average 79.6 d within a range from 77 to 84 d. In contrast, the breeding females of the zoo Dresden had very long inter-birth intervals ($\bar{\emptyset}$ 120.95 d; Ludwig and Krönke 2012). In addition, they were also significantly longer than in the colonies from the IZW (Mann-Whitney U-Test, $n=41$, $U=126.00$, $Z=-2.19$, $p=0.03$). They ranged from 81 to 274 d ($n=20$, $SD=58.67$) which is also highly variable.

As shown in this study, litters in which more than 50% or all pups died (L12, L13, L23) did not correlate with a significantly prolonged or short birth interval to the subsequent pregnancy. The reasons for long inter-birth intervals in NMR are largely unknown, however, there is a strong indication that various facts can influence the reproduction cycle of a queen. During this study only one mating was observed. This mating occurred on the 16th day after birth of the previous litter. This is only slightly longer than the 7 to 11 d observed by other scientists (Jarvis 1991; Lacey and Sherman 1991). Behavioral oestrus lasts for only 2 to 24 hr, during which repeated mating occurs (Jarvis and Bennett 1991). However, it has been observed that sometimes NMR pairs failed to successfully mate. Faulkes et al. (1990a) showed that the ovarian cycle of NMR with its long luteal phase lasts approximately 28 d. The inter-birth intervals may be prolonged,

if mating was unsuccessful by e.g. (i) disturbance of the mating, (ii) an inexperienced partner or (iii) false point of mating (during infertile days). In addition, if the conception was successful, also intrauterine factors may influence a pregnancy e.g. the ovulation without fertilized egg, occurrence of cysts on reproductive organs or total resorptions of the foetuses. Roellig et al. (2011) documented for the first time the occurrence of embryonic resorptions in NMR. It was shown that total resorptions in this species often lead to a uterine inflammation and possibly result in a delayed next pregnancy. This might explain the long parturition intervals of up to 274 d (Ludwig and Krönke 2012) especially common for the first pregnancies of a queen.

Litter size

In NMR colonies litter size seems to be highly variable, over all ages and body sizes of breeding females (Jarvis 1991). Only limited information is available on litter sizes of other NMR colonies. In the captive colonies of the IZW the mean litter size was 11.87 and ranged from 5 to 20 pups (Tab. 2). The average litter size was similar to that of captive colonies of the Vanderbilt University (Henry et al. 2007; t-Test, $n=41$, $t=0.86$, $p=0.40$). However, the average litter size of the captive breeding females of the IZW was significantly higher compared to wild NMR reported by Jarvis and Bennett (1991; t-Test, $n=49$, $t=4.79$, $p=0.00$).

NMR have the largest litters by far, compared to other non-domesticated species in the order Rodentia (Hayssen et al. 1993), including more than 15 species of the family Bathyergidae (Jarvis and Bennett 1991). With the exception of NMR, most other Bathyergids tend to have smaller litters (1 to 10 newborns) with one up to maximally four litters born annually (Bennett et al. 1991; Jarvis and Bennett 1991).

NMR Queens exhibit a small body size compared to other breeding females of the Bathyergids (Bennett et al. 1991). Breeding NMR females have a medium body weight of ~45 g and a body length of ~18 cm and produce extremely large litters. With an average newborn weight of 1.90 g (in this study) and a maximal recorded litter sizes of 28 pups in the field (Sherman et al. 1999) and 30 in captivity (R. Buffenstein, pers. comm. 2013), pregnant queens may increase her body weight by as much as 87% (Jarvis 1991). However, as noted by Jarvis and Bennett (1991), average litter sizes are commonly larger in captivity than in the wild. The frequently high litter sizes of captive NMR indicate the potential to bear more offspring under optimal conditions. In the wild, reproductive opportunities may be limited by e.g. food availability.

Interestingly, Buffenstein (2008) and O'Riain et al. (2000) reported significantly larger litter sizes by established breeding females than by newly established queens. In contrast, in captive colonies of the Vanderbilt University (Henry et al. 2007) as well as in the colonies of the IZW, no significant correlation was documented between litter size at birth between these groups or litter order or the numbers of previous litters (Spearman Rank Correlation, $n=22$, $r=-0.005$, $p=0.98$).

However, O'Riain and colleagues (2000) showed that established breeding NMR females exhibit significantly longer lumbar vertebrae in relation to their body size compared to remaining individuals of the colony. A pregnancy-induced mechanism leading to lumbar extension and reproduction-related bone growth is also described for many other mammal species. Research has shown increased bone metabolism and growth or density changes during gestation in humans, sheep, rats as well as in monkeys, as an adjustment for serving to concentrate mineral reserves that can be exploited for milk production and fetal skeletal development (Bowman and Miller 1999). Yet, the pregnancy-related bone growth in NMR queens differs from that of other mammals. Henry et al. (2007) showed that in NMR newly established breeding females developed an irreversible vertebral lengthening of 0.020 ± 0.012 mm/wk with each successive pregnancy. The body length of the three queens in the IZW was not measured during this study, as no vertebral lengthening in the queens was observed. The unique way of life of NMR may lead to this special pregnancy-related growth in the breeding females. The NMR queen is the only female individual within a colony that produces offspring, exerting sexual suppression through behavioral and hormonal cues towards the other female colony members. Due to the fact that NMR live in subterranean tunnels, a breeding females body size is limited by the inside diameter of the foraging tunnels (the diameters 35 to 45 mm; Jarvis 1991), which she has to walk through in order to maintain her position. Consequently, breeding females have to adapt to these environmental conditions, and evolution seems to have favored increased lumbar length as a mechanism for enable huge litter sizes without excessive enlargement of the body (Jarvis and Bennett 1991; O'Riain et al. 2000). The alternative, the extension of the tunnels to accommodate the gravid breeding female, would most likely require excessive metabolic costs.

Birth weight

Mean birth weight of all newborns in these colonies was 1.90 g and ranged from 1.05 g to 2.65 g (Tab. 2). Overall, the data did not differ from other captive colonies (Buffenstein 2008), nor from data observed in a colony of wild caught individuals (Jarvis et al. 1991). As already observed in other colonies (Jarvis 1991), in this study the body weight of newborn NMR seemed to be highly variable, even for the same breeding female and within one litter.

Buffenstein (2008) reported that in her colonies elderly breeding females produce heavier pups than young breeders. In contrast to Buffenstein, the oldest breeding female at the IZW, queen QI compared to the two other queens produced offspring with significantly lower birth weight (\bar{Q} 1.38 g). Queen QII** had the largest litters (Tab. 2), but also the heaviest newborns with a mean birth weight of 2.04 g. However, when averaging the litters of all three queens, the birth weight of the pups significantly decreased with increasing litter size (Fig. 11). Furthermore, Jarvis et al. (1991) detected no difference in sex ratio and birth weight between her colonies. In the colonies of the IZW, the male newborns were significantly heavier at birth than their female littermates.

Bennett et al. (1991) presented in his study that the birth weights in solitary mole-rat species range from 5.3 to 34 g and are generally higher than the birth weights of social living mole-rat species with 1.8 to 9 g (Bennett et al. 1991). Due to the wide interspecific variations in birth weight within the Bathyergidae, birth weight seems to be related to the social organisation of a mole-rat species. In solitary mole-rat species pups leave their natal burrows and disperse about 55 to 65 d after birth (Bennett et al. 1991). Therefore body weight at dispersal may be critical to the survival and favoring heavier moles-rats which may more successful in escaping predators and competing with other moles. In contrast, social mole-rats usually remain in their natal burrow and, in established colonies, will be supplied with food by the other colony members. As they can benefit from the care and protection of the other colony members, pups of social Bathyergids afford to develop more slowly.

Interestingly, although the social mole-rat species, such as the Damaraland mole-rat (*Fukomys damarensis*) and the NMR live in large colonies with brood care, newborns of NMR exhibit by far the lowest birth weight of all bathyergid mole-rats (Bennett et al. 1991). The main reason for this may be the large litter sizes of NMR. NMR queens exhibit a small body size (~18 cm) and

body weight (~35 g) and produce extremely large litters of up to 30 newborns (R. Buffenstein, pers. comm. 2013), twice as many as other bathyergids species (Bennett et al. 1991).

Growth of pups

In this study, only few continued data on the growth for NMR pups could be collected. The majority of born pups did not survive until weaning age in this study (Tab. 2).

Looking at the growth curves of NMR pups during this study period (Fig. 13), considerable differences in body weight between single days occur, also in successfully reared litters. NMR queens can produce litters of up to 30 pups (R. Buffenstein, pers. comm. 2013); the numbers of mammary glands on queens averaged 11.5 ± 2.0 (Sherman et al. 1999). This leads to a high competition for access to the teats within a litter and may lead to infrequent suckling opportunities and growth rates in the newborns.

Bennett et al. (1991) showed in his study that the two social mole-rat families compared to solitary mole-rat species exhibit a considerably slower average growth rate during the first 70 to 80 d of postnatal development. Additionally, the NMR pups also display the slowest average maximal growth rate compared to the other social mole-rat family *Cryptomys* (Bennett et al. 1991). Other studies on mammal species additionally provide evidence for a positive correlation between pre-/post-natal growth rates and basal metabolic rate (McNab 1980; Thompson and Nicoll 1986). The on average low growth rates in subterranean rodents are contrastable to values obtained for other comparably body sized subterranean and surf dwelling rodents (Bennett et al. 1991). Thus, the low growth rate of NMR may be a consequence of their subterranean life-style, comprising low basal metabolic rates and poikilothermy (McNab 1966; Withers and Jarvis 1980; Lovegrove 1986; Buffenstein and Yahav 1991). Interestingly, Brett (1991a) observed that wild juveniles grew faster compared to juveniles reared in captivity.

As previously shown by O'Riain and Jarvis (1998) and also confirmed in this study, already NMR newborns exhibit a wide variation in growth rate and body weight between and within the litters of the same colony (Bennett et al. 1991). Similar to the colonies at the IZW (Fig. 9), these NMR were also housed under laboratory conditions and libitum access to food. In this as well as in O'Riain and Jarvis' study (1998), all littermates were siblings from the same mother or at

least closely related. Given that NMR normally exhibit a high degree of inbreeding (Faulkes et al. 1990; Reeve et al. 1990; Honeycutt et al. 1991b), it is unlikely that differences in growth rate can be attributed to genetic differences. These facts provide support for the existence of a size-age correlation with social rank, such as in some other animals e.g. lions (Betram 1978) or dwarf mongoose (Rasa 1977). In 1995 (Schieffelin and Sherman) had assumed that there is also a positive correlation with body size and social rank in NMR.

As presented in this study, the body weight of NMR above an age of 80 d was extremely plastic and high variable, thus the body weight was not related linearly to the age of the moles. The possibility of using morphological information e.g. body weight or size to determine age was investigated by Hagen (1985), using 23 head and body measurements take on a captive NMR colony (n=71). This colony consisted of 29 NMR with known birth date. Using univariate and multivariate analyses, Hagen discovered a significant positive correlation between the height of the coronoid process of the lower jaw and the age of an animal. After stepwise reduction of variables, Hagen explored that the thickness and density of the lower incisor could also be used to estimate age but that the correlation was not as good as with coronoid height. Morphometric studies have often limited value in aging live animals, however, and the use of other methods for age determination (e.g. abrasion of the epiphyse, vertebrae and teeth, accumulation of lipofuscin in the skin) has to be investigated in NMR first.

Interestingly, O`Riain et al. (1996) discovered a new and rare phenotype within NMR colonies, the “disperser”. Dispersers are individuals that are characterised by a heavy body weight and large deposition of body fat, located especially in the neck. These NMR stemming from different litters, display different growth surges at different stages. Therefore, it is likely that not one single cue is responsible for triggering the change in body weight. Such variation in body weight between litters and the variable growth response of differently aged individuals under identical environmental conditions suggests that caution should be exercised when the body weight is used as an indicator of age in NMR colonies. In addition, a slow prenatal development has also been observed in some solitary mole-rat species e.g. the silvery mole-rat (*Heliophobius argentecinereus*; Sumbera et al. 2003). This fact may indicate that different growth rates in NMR cannot solely be linked to the social organisation in this species. Furthermore, considering the phylogenetic tree of bathyergids, NMR are on the base of it

(Deuve et al. 2008; Faulkes et al. 2010), assuming that these developmental traits might be an ancestral characteristic (Sumbera et al. 2003).

Surprisingly, in this study there was a slightly difference in growth rate between female and male pups of up to an age of 80 d postpartum (Fig. 13). However this difference in body weight of the gender leveled out when pups got older (Fig. 14). Also, no sex linked division of labour has been found so far in NMR colonies, thus the reason for the different growth rate of different sexes are still unexplored. In contrast, the second social bathyergid species, the Damaraland mole-rat (*Fukomys damarensis*) exhibits an obvious sex linked growth rate throughout their whole lifespan (Bennett et al. 1991). In the species *F. damarensis* males are substantially heavier than females, thus this appears to be partly the effect of sexual dimorphism (Young and Bennett 2013).

A further aspect of interest is that in this study a significant positive correlation was found between colony size and mortality rate of pups at an age of three months (Pearson Correlation, $n=23$, $r=0.42$, $p=0.05$). Also several other researchers found that the number of individuals in a colony seems to affect the rate at which the pups in subsequent litters grow (Bennett et al. 1991). O'Riain and Jarvis (1998) showed that if population density of a NMR colony was reduced, subsequent litters suddenly exhibited a distinct increased growth potential. Additionally, Young and Bennett (2013) demonstrated in their studies that the first litters born and raised by a pair of *F. damarensis* grew obviously faster (0.2-0.52 g/d) than those raised by small colonies (0.13-0.32 g/d). The same was observed in NMR pups (Bennett et al. 1991; Jarvis et al. 1991). It seems that litters born to a small colony have a higher chance to survive, for example due to a better access to the queen's teats in the nest, where lactating and huddling of the colony takes place. Further small colonies seem to invest more energy into caring for the pups. This accelerated growth of the first litter born to a new colony may reflect the need for the offspring to participate as soon as possible in colony work, e.g. it enables them to help with digging and foraging at an earlier age than those born to a more established colony. Thus, established colonies with a high number of individuals seem to have a higher selective pressure compared to small, newly established colonies.

Sex ratio

In the past it was generally thought that in mammals the sex ratio of male and female offspring is completely random. However, in the last years many researchers have shown for several different species, that sex ratio of the offspring may be significantly biased (Saragusty et al. 2012).

In contrast to almost all other Bathyergidae species, NMR exhibit neither sexual dimorphism including the absence of differences in external genitalia (Fig. 7). Thus, to detect the gender and to determine the sex ratio in NMR, it is necessary to perform molecular techniques e.g. fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR). The research group Reproduction Management of the IZW tried to establish the cytogenetic technique FISH by using ejaculate of NMR, however FISH is not a properly working method in NMR yet (T. Hildebrandt, pers. comm. 2014). The biochemical technique PCR to determine the sex ratio in NMR works well (Fig. 10), but the first molecular sexing protocol for this species was published only in 2010 by Katsushima et al. Because the method was published only some years ago, until now just few sex ratio data using this trustful approach are available. Therefore, outdated literature has to be considered as critical. Due to the high incidence of errors using a visual sexing method based on external traits, it is not surprising that in the literature contradictory sex ratio data for NMR is found. Jarvis (1991) noted a sex ratio of 1:1 in her captive born NMR colonies, whereas she found in the wild (Jarvis 1991) and other captive colonies (O'Riain and Jarvis 1998) a male shifted sex ratio at birth. Interestingly, apart from the method which was used, all researchers found a sex ratio moved towards males in both - captive and wild adult NMR (Jarvis 1985; Braude 1991; Brett 1991a; Sherman et al. 1992; Nigel and Faulkes 2000).

In the captive colonies of the IZW sex ratio at birth was slightly male shifted 56% (n=245; Tab. 2; Fig. 17). Available sex ratio data from litters included in this study of one or two years as well as from older litters showed that the male biased sex ratio remained unchanged for all life stages. However, differences in sex ratio were observed considering individual litters of each of the three queens (Tab. 2; Fig. 17). Overall, the male shifted sex ratio in the NMR colonies at the IZW is a non-significant trend (Tab. 2). More data of captive colonies are needed to determine whether NMR colonies are significantly male skewed or not.

Nevertheless the mechanism and factors influencing the sex ratio in NMR are still largely unknown. A variety of aspects e.g. population density, dispersal, predation, hierarchical

struggles or also intrauterine mechanisms of the breeding queen may have an effect on the sex ratio in the NMR colonies.

It is assumed that social stress by i.e. high population density or hierarchical disagreements of individuals may have an influence on the sex ratio of pups by e.g. intrauterine resorption, cryptic female choice or biased sex ratio in the ejaculates. Roellig et al. (2011) documented for the first time the occurrence of embryonic resorptions in NMR, but it is still unclear which other factors might influence the sex ratio in NMR. At the IZW, the colony I and II was established with a small colony size with less than 27 members each, the first litters of QII* (L14-L16) and QI (L3) were female shifted (Tab. 2) even if there was already a high proportion of females in the colony. Surprisingly, after the third litters of both queens all further litters were male shifted (Tab. 2). After one year of regency and four successful litters in colony II, queen QII* was killed by another female - queen QII**. In contrast to the two other queens, queen QII** started her regency in an established colony with more than 30 colony members (~14 females, 17 males). All litters of queen QII** were male shifted from the beginning (Tab. 2). 80% of the litters in the colonies of the IZW were male biased at birth (Tab. 2) and consequently these NMR colonies exhibit more male than female colony members. Thus, in this study population density or sex ratio of the NMR colony had no clear effect on the sex ratio of the newborns. But more data of other colonies should be analysed to evaluate if population density or sex ratio of a colony may have a significant influence on the sex ratio of the offspring or not.

Intersexual variations in dispersal behavior may also explain the skewed adult sex ratios apparent in other subterranean species. Due to the fact that in most mammals mainly males disperse and mortality during dispersal is high, it is not surprisingly that almost all Bathyergidae species are female biased e.g. Damaraland mole-rat (*Fukomys damarensis*; Jacobs et al. 1991), Common mole-rat (*Cryptomys hottentotus*; Begall and Burda 1998) and Ansell's mole-rat (*Fukomys anselli*; Sichilima et al. 2011). This explanation may not hold for the NMR. In NMR dispersal also occurs, but extremely rarely (O'Riain et al. 1996). Braude and Cizek (1998) reported that only one of 7.000 mole-rats is known to have migrated from one colony to another during the 11 yr of their study. Additionally, even if dispersal in NMR seems to be strongly male biased (O'Riain et al. 1996), also females have been observed to disperse (Nigel and Faulkes 2000). Another possibility may be that especially female NMR are engaged in tasks that expose them to a higher risk of predation e.g. pushing soil out of the burrow system.

However, there is no evidence supporting the idea of a gender-dependent division of labour in NMR yet.

The maintenance of the NMR hierarchy in a colony often led to fighting between the queen and other large, dominant individuals. In particular other dominant females, who want to replace the queen, are the primary victims of the queen's aggression. The experience of captive colonies shows, that if aggressions escalated, this often results in the death of one or more adult NMR. If this happens repeatedly, it may be skew the sex ratio of the colonies towards males (Jarvis 1991; Brett 1991a). However, observation in the past showed that during hierarchy struggles also adult male died (Clarke and Faulkes 2001). Additionally, once a hierarchy is established with a queen in a colony, the NMR queen may breed for over than 15 yr (Buffenstein and Jarvis 2002) without any deadly conflicts occurring in the colony during this entire time span.

For other cooperatively breeding species, the most common, used and intensely discussed explanation for sex ratio variation is the "helper repayment hypothesis" (Gowaty and Lennartz 1985; Emlen et al. 1986; Lessells and Avery 1987). Based on other cooperatively breeding species such as the african wil dog (*Lycaon pictus*), the red-cockaded woodpecker (*Picoides borealis*), cichlid fish (*Neolamprologus pulcher*), alpine marmots (*Marmota marmota*), meerkats (*Suricata suricatta*), Florida Scrub Jays (*Aphelocoma c. coerulescens*) and marmosets (*Callithrix jacchus*) or this hypothesis predicts an overall sex ratio bias towards the gender which helps to improve the breeder's live time reproductive success. Among cooperative breeding species in general, and birds in particular, the males commonly are generally more philopatric, whereas females are more incline to dispers (Rowley 1964; Zahavi 1974; Gaston 1978). Thus, it is not surprising that among other socially monogamous and cooperatively breeding mammals e.g. the Mongolian gerbils (*Meriones unguiculatus*; Agren et al. 1989) or the Common mole-rat (*Cryptomys hottentotus*; Genelly 1965) the males are responsible for rearing the offspring and the adult sex ratio is male biased in these species. Even though so far very little is known about a potential sex-linked division of labour in NMR, first data collected in the colonies of the IZW indicate that proportionally males are more involved in caring for the offspring than females. Thus, this fact may explain why a higher proportion of males can be found in the colonies of this study.

Another aspect of gender distribution is that breeding males do not seem to have much influence regarding the sex ratio of their offspring as it was contrary reported for pygmy hippos (*Choeropsis liberiensis*; Saragusty et al. 2012). In colony II, QII* was killed during a colony struggle, but the breeding male survived. The successive, new queen QII** also accepted this male as her new breeding male. This breeding male produced almost exclusively female-biased litters with the first queen QII*, whereas the litters of him and the second queen QII** were strongly male biased (Tab. 2).

Mortality

The presented data show a quite high mortality of NMR pups in the colonies of the IZW (Tab. 2; Fig. 18). Although more than 58% of the 273 newborns died during their first weeks of life, the mortality rate was lower than in other NMR colonies. In 52% of the litters more than half of the offspring survived at the IZW. Jarvis (1991) reported a highly variable weaning success (14% to 52%) in her colonies, too. A high pup mortality rate seems to be a common and frequently observed phenomenon in captive NMR populations (Jarvis 1991; Lacey and Sherman 1991; Petry 2003). To date nothing is known about the survival of pups in the wild nor the reason for this high pup mortality in captivity.

Due to the fact that in this study all pups of the most recent three litters died during the first two weeks of their lives and a significant positive correlation between colony size and pup mortality was found, overpopulation in these colonies could be the cause for this correlation. Roellig et al. (2011), Jarvis (1991) and Buffenstein (2008) also supposed that rapid colony growth contributes to high postnatal pup mortality as a consequences of a density dependent increased selection pressure. Consequently, high population density might favor the death of NMR pups.

In contrast to the findings of Buffenstein (2008), in this study larger litters did not significantly cause higher pup mortality. However, in both studies increasing age of the queens was positively correlated with the ratio of dead offspring. This statement of Buffenstein (2008) is based on a long-term study comprising very old breeding females (>20 yr) with a reproductively active period of more than 11 yr. Buffenstein (2008) concluded that elderly breeding females fail to raise their young, because they cease to exhibit an adequate maternal milk production. This explanation does not apply to the breeding females of the IZW. Even if the exact ages of

these breeding females are unknown, all of them are younger than 10 yr and have been breeding for maximally one to four years. However, possibly another factor influenced the mortality of the pups at the IZW and also caused the age-dependent bias. At the beginning of 2012 extensive renovation work began at the IZW, accompanied by loud noise and strong ground vibrations. Considering that NMR possess a highly developed vibration sense as an adaptation to their subterranean lifestyle (Quay 1981), the animals are extremely sensitive to the latter (Brett 1985; Jarvis and Bennett 1991; Lacey et al. 2000). In nature, some predators of mole-rats, e.g. sand boas (*Eryx colubrinus*) or the rufous-beaked snakes (*Rhamphiophis oxyrhynchus*) have been found in NMR burrows (S. Braude, pers. comm. 2013). Even though it is not well-documented how often disruptions of a colony occur in the wild, the fact that NMR typically dig 'bolt holes' (dead end tunnels) that pass downwards from the communal nest (Brett 1991) points out that emergency escape from the nest box is sometimes essential. Captive NMR responses to disturbances of their nest (e.g. daily cleaning) may simulate what occurs in the wild. In captivity, when disturbed, NMR become extremely frightened and abandon their communal nest chamber, especially when they have newborns or young pups (Ludwig and Krönke 2012). In colonies containing newborn pups, the other colony members always try to "save" the offspring and carry them out of the nest and through the whole burrow system (Jarvis 1991) when disturbed. If the perturbation lasts too long or is too frequent the behavior of the colony changes; less care is given to the pups which often results in their death (Jarvis 1991; Ludwig and Krönke 2012). Stressed breeding females reduce their maternal care, e.g. do not permit her pups to suckle anymore (Jarvis 1991; Ludwig and Krönke 2012). Pups are being insufficiently warmed, pushed to the edge of the nest chamber or are not returned if they are dislocated from the nest. Moreover, adult NMR exhibit a frequently observed but unusually aggressive specific behavior towards the pups: they shove the pups frequently and vigorously with their head or mouth inside as well as out of the nest. The pushing usually is repeated in rapid succession such that a pup is often moved for a distance of up to 15 to 20 cm away (Lacey et al. 1991). As - at least occasionally - this behavior has been observed in every colony and litter so far studied in captivity (Jarvis 1991; Lacey and Sherman 1991; O'Riain et al. 1996), it implies that it is a normal aspect of juvenile development. However, the behavior increases dramatically when colonies are stressed resulting in weakened pups. In addition, considering the development of body weight particularly of litter L12 in colony I (Fig. 16A), the pups did not

simply die of starvation. Rather their weight increased and decreased during a period of two weeks until they died. These weight curves obviously differed from a normal development (Fig. 16B), which supports the hypothesis that the extensive renovation work at the IZW had a considerable negative influence on the survival rate of the pups. Moreover, the last litter (L23) of queen QII** was unusually small (five pups) and all babies were born dead (Tab. 2). Stillbirth in NMR is quite unusual and very rare. In the survival of litters born before and after the time of the renovation work at the IZW, a significant increase in mortality rate has been observed during the construction work (Tab. 2). Taken together, even if the reasons for the high infant mortality rate in NMR are not sufficiently investigated yet, NMR colonies seem to have a very complex and changeable social system, which may respond more sensitively to external influences than previously assumed.

Interestingly, 54% of the dead pups simply disappeared and were not detectable anymore. As the retrieved dead newborns often exhibited traces of consumption, NMR obviously cannibalise. Cannibalism is common in many species in both terrestrial and aquatic habitats (Elgar and Crespi 1992) e.g. sharks, birds, arthropods, reptiles, fishes, amphibians and mammals. In NMR this behavior has previously been described for several other captive colonies (Jarvis 1991; Lacey et al. 1991; Buffenstein 2005; Ludwig and Krönke 2012). Also the second social mole-rat species, the Damaraland mole-rat (*Fukomys damarensis*) was observed to consume dead offspring (Bennett and Jarvis 1988b). In other rodents such as the Syrian hamster (*Mesocricetus auratus*; Beery and Zucker 2012) or the white-footed mouse (*Peromyscus leucopus*; Wolff and Cicirello 1989), it is often just the breeding or lactating female, who feeds on the offspring. Based on observations during this study, all members of the colony consumed dead newborns. Furthermore, in this study the breeding females, queen QI and QII** were observed not only eating dead newborns, but also living pups. So far it is not known whether infanticide is a frequent behaviour in NMR or rather an uncommon act. In any case it is a rarely mentioned fact, and has only been published for the colonies at Cape Town (Jarvis 1991). The causes for cannibalism in mammals are diverse and depend on the circumstances. As protein sources in subterranean burrows are limited, NMR may eat their offspring because of a protein deficiency. However, considering that NMR also practise infanticide, it further also seems to be a mechanism for selection.

In addition, Jarvis (1991) reported that fewer males than females died before weaning and therefore the mortality rate in her colonies was male-biased. Although the sexing was confirmed by autopsy, the study considers only the retrieved dead pups and not the eaten ones which is a critical aspect. In the present study no significant difference in pup sex and time of death was detected.

5. Conclusion and outlook

NMR are one of the most fascinating and extraordinary mammals that are known. Due to their special characteristics, they are often kept and investigated in zoological institutions and research institutes worldwide. But so far fundamental questions about NMR are still unclarified, even though there is a comprehensive research on these moles for many years. For example, basic facts about reproduction in NMR are well documented, but successful breeding and rearing of offspring is still very difficult in captivity. The experiences about breeding in NMR gathered over the last four years within this study may be helpful and useful to enhance the breeding success of other captive colonies, too. The received results of pup ontogeny and reproduction may also help to extend the basic knowledge about NMR and can be beneficial for future research questions such as how their complex social system is build up, how it functions and how it develops at the beginning of a colony or if there is an increasing selection pressure to the survival rate of newborns in established colonies compared to newly-established colonies. However, to answer these questions more long-term investigations will be require.

6. References

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Die Arbeit wurde bisher weder im In- noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.“

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2013 HOLTZE S, MORHART M, DEHNHARD M, LUDWIG A, HILDEBRANDT TB (Poster): Investment in survival: Healthspan extended- the naked mole rat case. Evaluierung des Leibniz- Institutes für Zoo- und Wildtierforschung (IZW), 13.-14.06.2013, Berlin/G.
2013 MORHART M, HOLTZE S, HILDEBRANDT TB (Poster): Pup ontogeny in naked mole-rats. Nothing but a rat race? 9. International Conference on Behavior, Physiology and Genetics of Wildlife, 18.-21. September 2013, Berlin/G.

- 2014 MORHART M, HOLTZE S, HILDEBRANDT TB (Poster): Nothing but a rat race? Pup ontogeny in naked mole-rats. 88th Annual Meeting of the German Society for Mammalian Biology, 01.-04. September 2014, Giessen/G.
- 2015 MORHART M, HILDEBRANDT TB (Talk): Parturition and postnatal care in a eusocial rodent, the naked mole-rat (*Heterocephalus glaber*). The 10th topical meeting of the Ethological Society, 11.-14. February 2015, Hamburg/G.
- 2015 MORHART M, HILDEBRANDT TB (Talk): New insights into postnatal development-, growth pattern- and behavior in a eusocial rodent, the naked mole-rat (*Heterocephalus glaber*). The 12th African Small Mammal Symposium, 12.-18. April, 2015/Mantasoa (Madagascar).
- 2015 MORHART M, HILDEBRANDT TB (Poster): Birth and postnatal care in a eusocial rodent, the naked mole-rat (*Heterocephalus glaber*). The 12th African Small Mammal Symposium, 12.-18. April, 2015/Mantasoa (Madagascar).

Populärwissenschaftliche Beiträge

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- 2012 Nacktmulle tanzen aus der Reihe. Menschen, Tiere & Doktoren, VOX, 03.11.2012.
- 2013 Lebenslang gesund dank hässlichem Nager? Einstein, SRF, 24.10.2013.
- 2014 „Das hässlichste Tier der Welt“- Der Nacktmull. X:enius, ARTE, 14.01.2014.