universität wien

MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

„Non-invasive bodyfat calculations in Common hamsters (Cricetus cricetus) using morphometric measurements "

verfasst von / submitted by

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angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien, 2016 / Vienna 2016

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	A 066 878
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Masterstudium Verhaltens-, Neuro- und Kognitionsbiologie
Betreut von / Supervisor:	Ao. Univ.-Prof. Dr. Eva Millesi

Danksagungen:

Zuallererst möchte ich mich bei Ao. Univ.-Prof Dr. Eva Millesi bedanken für die Möglichkeit zu dieser Masterarbeit sowie ihre Betreuung und Geduld.

Ich möchte mich auch bei Carina Siutz für ihre Hilfe in statistischen Fragen bedanken und für die unzähligen Telefonate sowie den email Verkehr.

Ich möchte mich an dieser Stelle auch noch bei den Kolleginnen und Kollegen des Departments für Verhaltensbiologie bedanken, welche mir meine Pausen versüßt haben. Desweitern wollte ich mich ganz herzlich bei meinen Freunden (Verena, Arthur, Sandra, Johanna, Caro und Christian) des Masterstudenten - Kämmerchens, mit welchen ich über alles reden konnte und durch die ich motiviert wurde vice versa, bedanken.

Auch den Mitarbeitern des Chemielabors des FIWI Minh Hien Le, Steiger Eva und Hämmerle Michael, welche mir mit Rat und Tat beistanden und mit welchen ich eine sehr informative, spannende und lustige Zeit verbringen durfte.

Ein großer Dank gilt natürlich auch meinen Eltern die mir nicht nur durch finanzielle Unterstützung geholfen haben, sondern auch durch ein offenes Ohr eine große Stütze waren in dieser Zeit. Meinen Geschwistern gilt ebenso großer Dank ebenso auch meinen Freunden, denen ich zum Teil das Ohr abgekaut habe aufgrund unterschiedlicher guter oder weniger guter Momente während der Arbeit. Besonders wenn ich ein und dieselbe Problematik immer und immer wieder bereden musste. Nochmals Danke das ihr alle für mich da wart!!!

Table of content

1. Abstract

Animals employ a variety of strategies to survive the winter including migrating, reducing their activity levels or hibernating. The common hamster (Cricetus cricetus) is a facultative hibernator combining the accumulation of external and internal energy reserves to overcome the winter. The aim of this study was to adapt an existing non-invasive method to calculate the proportion of body fat not only in adults and subadults but also in juvenile individuals. For this purpose, the body fat content of hamster carcasses was measured using Soxhlet lipid extraction and morphometric parameters like the hamsters' weight as well as head, tibia and foot length were combined in a statistical model to predict the proportion of body fat. In the second part of the thesis, field data were analyzed. We compared body mass, the proportion of body fat and the size parameters between males and female juveniles as well as between pups from litters born early and late in the season. We chose four phases, at natal emergence, two and four weeks thereafter and shortly before immergence into the hibernacula in autumn. A sexual dimorphism became evident already four weeks after natal emergence. Males had a higher body mass than females, which was mainly reflected in higher structural growth rates while the proportion of body fat did not differ between the sexes in this period. Shortly before hibernation female juveniles even had higher proportions of body fat than males. These differences could be explained by the high intrasexual competition among males during the mating period enabling only large males in good body condition at vernal emergence to reproduce successfully. Juveniles born late in the season were heavier than early-born ones after 2 weeks postemergence probably because of the smaller litter sizes later in the season and correspondingly more and perhaps fatter milk per pup. Shortly before winter early- and thus older juveniles were larger and heavier than the others, while relative body fat did not differ between the groups. This indicates that hamsters born early in the season use the temporal benefit mainly for growth to increase their chances for reproduction in the following season. For late born pups overwinter survival might be more critical and thus energy reserves are highly important.

2. Introduction

Body fat plays an important role for animals with regard to growth and energy storage for critical periods, particularly in regions with seasonal climate and temperatures below zero degrees during winter times (Humphries et al., 2003). Several animals have developed strategies to overcome the winter, such as the migration of birds into their overwintering habitats (Newton, 2008), lower activity rates and the reduction of organs in roe and red deer or hibernation as an efficient way to save energy (Arnould et al., 2015). There are two different types of hibernatiors in small mammals: obligate and facultative hibernatiors (Davis, 1976; Harlow & Menkens, 1986). The hibernation phase consists of alternating deep torpor bouts and short arousal episodes (Wassmer & Wollnik, 1997). Obligate hibernators, like some ground squirrel (*Spermophilus sp.*) and marmot species (*Marmota sp.*), have to gain sufficient amounts of body fat during the short summer period to survive the hibernation phase which lasts for several months (Ramos-Lara et al., 2014; DeCoursey, 2004). In contrast, the facultative hibernators, in our study the Common hamster (*Cricetus cricetus*), show high variation in their overwinter behaviour (Wassmer & Wollnik, 1997). Hamsters prepare for hibernation by combining (1) external energy reserves, which involves collecting and storing food items until autumn, and (2) internal energy reserves, by accumulating body fat prior to hibernation (Humphries et al., 2003; Niethammer, 1982; Weinhold & Kayser, 2006). Recent results indicate that adult female hamsters hibernate for shorter periods than males and thus depend more on external energy reserves compared to males (Siutz et al., 2016).

These ground dwelling rodents have to accomplish this task throughout their active season of 6 months (Franceschini-Zink & Millesi, 2008). Vernal emergence in these solitary animals can vary between years and regions (Weissinger, 2013;Weinhold 1998). Generally, male Common hamsters emerge from hibernation in early April, a few days before the females (Franceschini et al., 2007). During our study period, the first hamsters emerged even during March (Pluch, 2013; Weissinger, 2013). Males have to activate their reproductive system to be ready to search for females in oestrus as soon as they emerge. To do this they have to cover a large home range and mate with as many females as possible. The mating phase starts immediately after emergence of the first females (Franceschini-Zink & Millesi, 2008). As both males and females have been observed to mate with more than one partner the mating system can be described as promiscuous (Weinhold & Kayser, 2006). After parturition, the females can have a postpartum oestrus and thus become pregnant while lactating the current litter (Siutz et al.,

2012; Franceschini-Zink & Millesi, 2008; Pluch et al., 2013). Moreover, the common hamster can have up to three litters per season (Seluga et al. ,1996) with 1-9 pups (Franceschini et al., 2007). This is uncommon in hibernating small mammals, which usually have only one litter per season (Humphries et al., 2003).

If breeding was successful, the first litter emerged after a gestation period of 17 to 37 days, and another three weeks of lactation (Siutz & Millesi, 2012; Seluga, 1996; Niethammer, 1982; Weinhold & Kayser, 2006). The first litters usually emerge in May. Shortly after natal emergence the pups are weaned and disperse. The litter size of the first litter per season is usually higher than that of later ones and at the end of the season offspring of first litters has been shown to be heavier than later born juveniles (Hufnagl et al., 2011). This is not surprising as early born litters have more time to grow, find or build a new burrow, and accumulate more body fat reserves than late born ones (Franceschini-Zink & Millesi, 2008). The last litters left the burrows during August and all pups immerged into their hibernacula during October/November (Franceschini & Millesi, 2005; Siutz & Millesi, 2012). Former studies have shown that the survival rate of late born juvenile individuals is lower than in adults, probably because those individuals are not able to sufficiently prepare for the following winter (Seluga, 1996).

Although both female and male juveniles are capable of reproduction in their first season, it is rather uncommon. Most hamsters start to successfully reproduce as yearlings, although there are some cases in which juvenile females have been documented to reproduce within their first year (Franceschini et al., 2007; Franceschini-Zink & Millesi, 2008; Niethammer, 1982; Siutz & Millesi, 2012).

Adult males terminate above ground activity during September and immerge into their hibernacula shortly thereafter, preceding females (Hufnagl et al., 2011; Franceschini & Millesi, 2005). Common hamsters show high individual variation in body temperature patterns during hibernation, which were investigated in free-ranging hamsters (Siutz et al. 2016), under semi natural conditions (Wassmer, 2004) and in climate chambers (Siutz et al. unpublished data). The mentioned variation may be caused by differences in individual proportions of body fat and/or the availability and quality of food stores. Particularly in field studies information on food caches is lacking but calculations of body fat proportions in free-ranging hamsters could help to understand the high variation in the use of the energy-saving torpor state.

There are different methods to calculate body fat including isotope dilution, energy X-ray absorptiometry (DXA) or total body electrical conductivity (TOBEC). These methods are accurate, yet complex, expensive and require special equipment or an abundance of subjects (Tidhar & Speakman, 2007). In addition they cannot, or only with high limitations, be used in field studies. Hence, we aimed at developing a method to calculate the proportion of body fat using morphometric measurements, with parameters that can easily be obtained in the field, such as body mass, tibia, head and foot length.

This technique was already successfully applied by Siutz et al. (2012) with a lower number of subjects. The model developed by Siutz et al. (2012) allowed to predict the proportions of body fat in adult and subadult Common hamsters.

The aim of this study was to extend and validate this non-invasive model to be able to calculate body fat proportions also in juvenile common hamsters using morphometric parameters (body mass, head-, tibia- and foot length). Furthermore we compared proportions of body fat in free-ranging juvenile hamsters and compared body mass, body fat and parameters indicating structural growth between the sexes and between juveniles born early and late in the season.

3. Methods and Material

3.1. Study Animals

The non-invasive calculation of body fat proportions in common hamsters was previously validated based on lipid extraction of carcasses (n=11) and measurement of morphometric parameters, which were combined in a multiple regression model (Siutz et al., 2012). To improve the accuracy of the model and hence, the predictability of body fat proportions, we repeated this analysis with a higher sample size. We used 72 intact carcasses for analysis, which were provided by Chronobiotron laboratory breeding stock, Strasbourg (Authorisation B-67-482-25). Before they were killed using an overdose of isofluran they were singly housed in Makrolon cages type 3 and got pellets and water ad lib. None of the hamsters was killed for the purpose of this study. The age of the carcasses ranged between 0.6 and 16.4 months, body mass ranged from 55g – 559g. The hamsters were divided into two age groups, juveniles (32 females and 8 males) and adults (20 females and 12 males).

3.2. Body fat extraction

The carcasses were minced using a commercial meat grinder after body mass (g), head (mm), tibia (mm) and foot length (mm) had been measured. For lipid extraction we used Soxhlet technique (Jensen, 2007; Sukhija & Palmquist, 1988) with petroleum ether as solvent. After 6 hours the body fat was solved and weighed. Total body fat was calculated as percentage of total wet mass.

3.3. Field study

Field data from capture-mark-recapture studies in 2010 (Pluch et al. 2013) and 2012 (Weissinger, 2013) was used. Data was collected in an urban area in Vienna from a free-ranging population of common hamsters. In both years, the animals were captured using tomahawk live traps, marked and released. Sex, age, reproductive state, litter size were recorded and body mass, head, tibia and foot length were measured (Pluch et al. 2013; Weissinger, 2013). Birth date was determined by monitoring adult females reproductive status, rapid body mass loss and teat size, partly milk remains (Siutz & Millesi, 2012).

3.4. Dates and Phases

Natal emergence was defined as the date when the juvenile left the breeding burrow for the first time. Measurements of individuals were used for analysis when they were captured within two days after natal emergence. Further comparisons were done two weeks (± 2 days) and 4 weeks (± 2 days) after natal emergence.

The onset of the prehibernation phase in juvenile hamsters was at September 12th in both years. The last capture of the hamster after September 12th was defined as immergence date (Siutz et al., 2012).

Early born juveniles were born between April and mid-June and late borns between July and August

3.5. Statistics

The software R 2.9.2 (R Development Core Team 2009) was used for body fat analyses. To predict body fat proportions we performed a multiple regression model using the percentage of measured body fat as response variable and body mass, head, tibia, foot length, and their 2-way-interactions as predictor variables.

Subsequently, field data from juveniles of the years 2010 and 2012 was used to calculate proportion of fat and add it to the comparison of sex and early and late born juveniles. The year 2010 and 2012 were pooled after testing for potential year effects. For these calculations, the statistics software SPSS 23 was used.

All groups (sex and early –late) were tested for normal distribution with Shapiro-Wilk test. To compare groups (males vs. females and early- vs. late-borns), Student's t-tests for normally distributed and Mann-Whitney-U-tests for non-normally distributed data were used.

4. Results

4.1. Body fat proportions in carcasses

The body fat proportions of the carcasses were compared between sex and age groups (juvenile and adult). The females in each age group had higher proportions of body fat than the males (Fig. 1, Fig. 2). The difference was even more pronounced in the juvenile than in the adult age class.

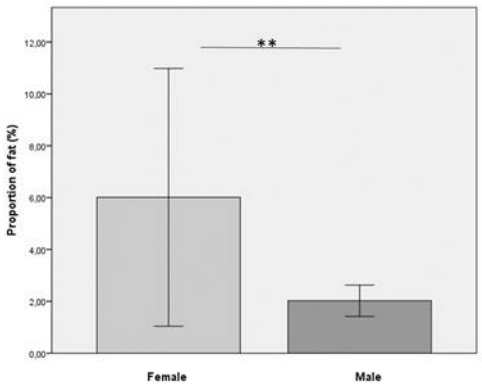

Fig.1. Proportions of body fat (%) in juvenile female and male hamsters (mean ± SD; females n=32, males n=8 p=0.007)

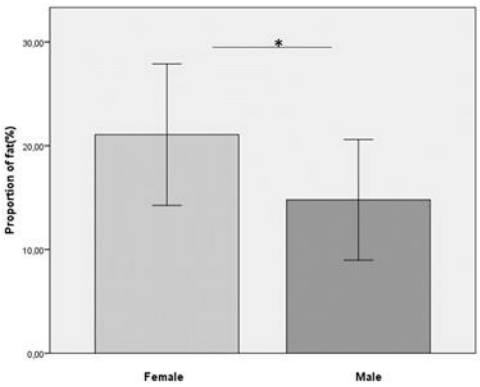

Fig.2. Proportion of body fat (%) in adult female and male hamsters (mean ± SD; females n=20, males n=12, p=0.012)

Age comparisons showed that in both sexes adult individuals had significantly higher body fat proportions than juveniles (Mann-Whitney-U-Test, $p<0.001$ in females and in males, Fig. 1, Fig. 2).

4.2. Validation of body fat prediction

The predictor variables body mass, head, tibia, foot length and their 2-way-interactions significantly affected measured body fat proportions and explained 96% of the variance ($F_{10,62}$=192.6, p=2.2x10^{-16}, R^2=0.96, n=72). By applying this model, body fat proportions could be predicted with a mean absolute error of 1.86% from measured values (Pearson correlation: r=0.96, n=72; Fig. 3).

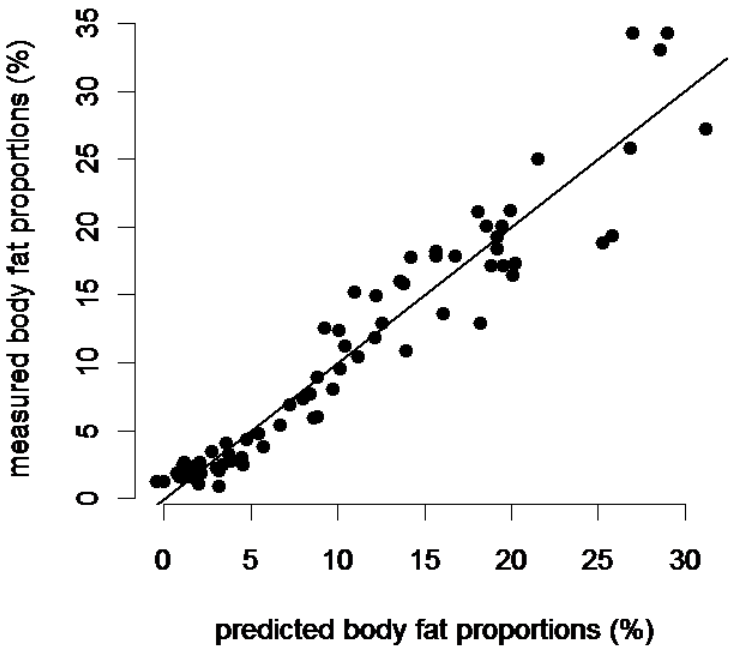

Fig.3. Predictability of body fat proportions based on a multiple regression model using measured body fat proportions as response and body mass, head, tibia and foot length as predictor variables (mean absolute error of predicted from measured values: 1.86%, Pearson correlation: r=0.96, n=72)

4.3. Field data

We found no significant sex differences in body mass of juvenile hamsters at natal emergence (Fig.4.) and two weeks later (Fig. 5). After 4 weeks post emergence, however, males were significantly heavier than females (Fig.6). This difference continued until the prehibernation phase. Males had a higher body mass shortly before immergence into their hibernacula than females (Fig. 7).

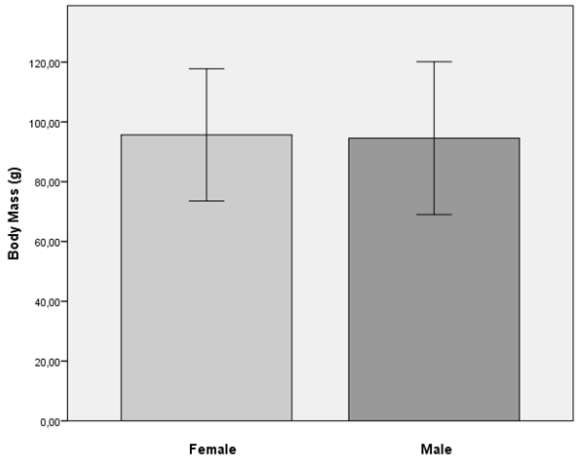

Fig.4. Comparison of body mass at natal emergence between juvenile females and males (means ± SD, females n=44, males n=56, p=0.721).

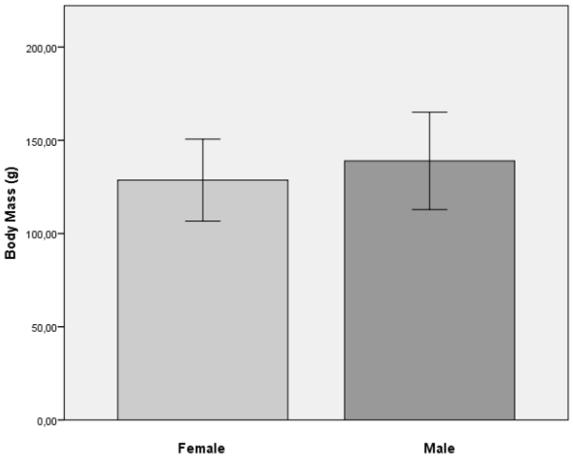

Fig.5. Comparison body mass between juvenile females and males two weeks after natal emergence (means ± SD, females n=29 males n=33, p=0.171).

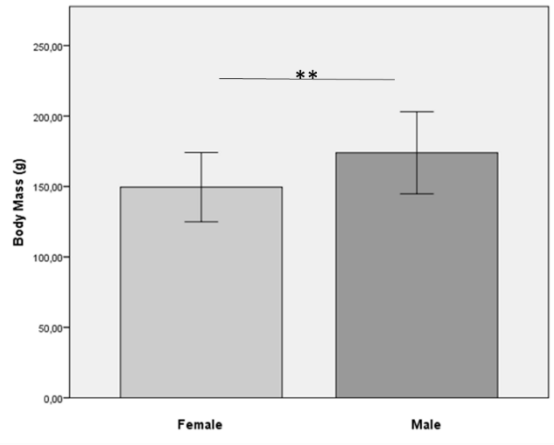

Fig.6. Comparison body mass between juvenile females and males four weeks after emergence (means ± SD, females n=21 males n=28, p=0.003).

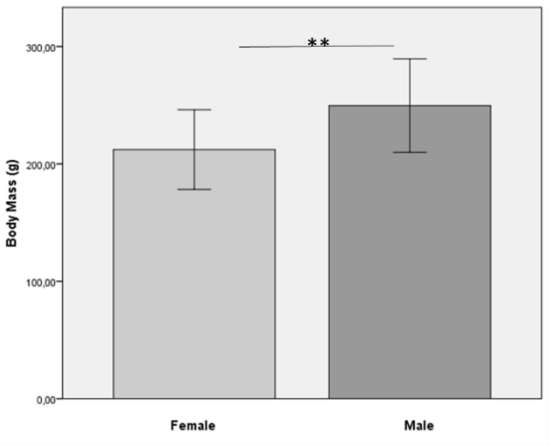

Fig.7. Comparison body mass between juvenile females and males at prehibernation (means ± SD, females n=13 males n=27, p=0.009).

We found no significance sex difference in the proportion of fat (%) at emergence (Fig.8.), 2 weeks post emergence (Fig.9.) and 4 weeks post emergence (Fig.10.). At prehibernation however females had higher body fat contents than males (Fig.11.).

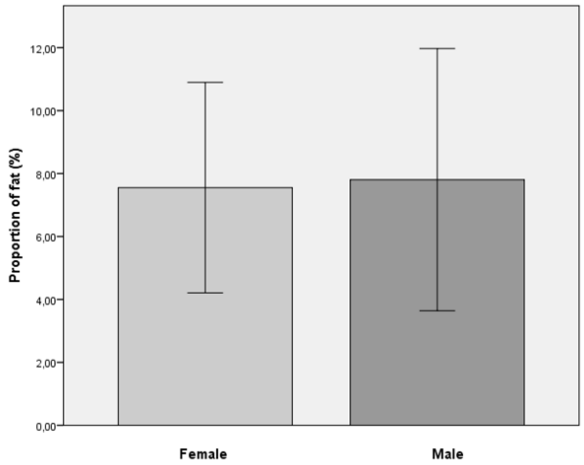

Fig.8.

Proportion of fat (%) at natal emergence in juvenile females and males (means ± SD, females n=44 and males n=56, p=0.797).

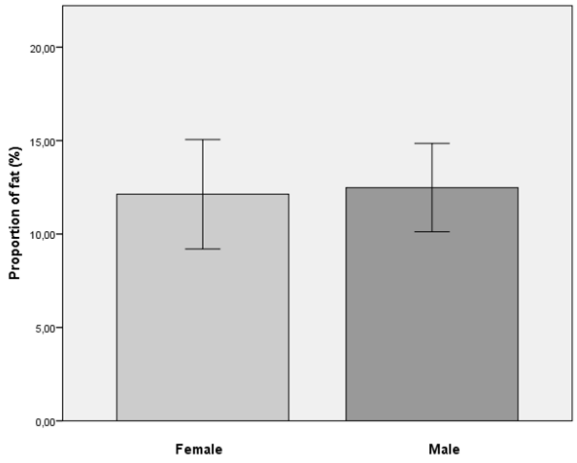

Fig.9.

Proportion of fat (%) two weeks after emergence in juvenile females and males (means ± SD, females n=29 and males n=33, p=0.657).

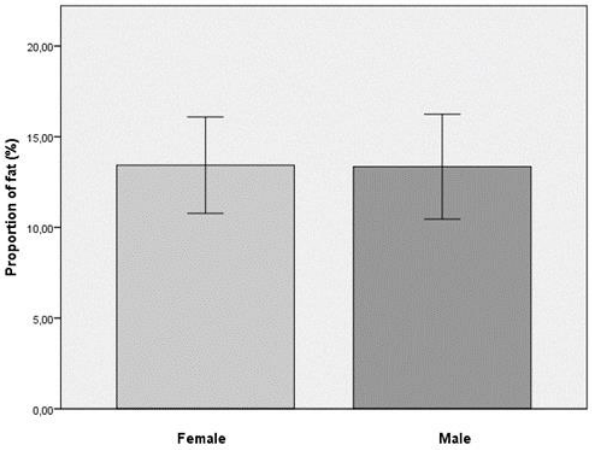

Fig.10.

Proportion of fat (%) between juvenile females and males four weeks after emergence (Mean ± SD, females n=21 and males n=28, p=0.747).

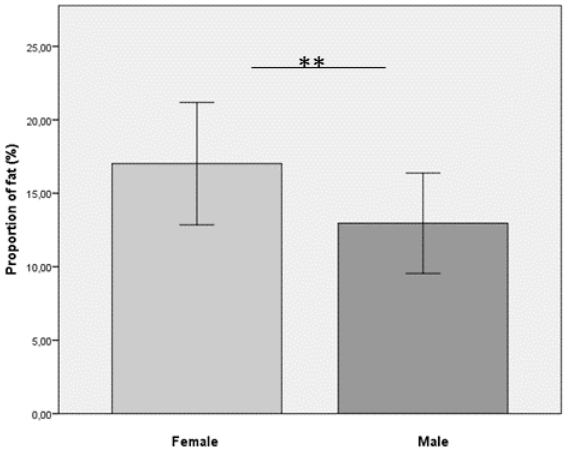

Fig.11.

Proportion of fat (%) at prehibernation in females and males (mean ± SD, females n=13 and males n=27, p=0.007).

Head size did not differ between the sexes at natal emergence and two weeks later (Tab.1). Four weeks post emergence males had significantly larger heads than females. At prehibernation, however, we found no significant differences between males and females (Tab.1).

Tibia length did not differ between the sexes at natal emergence, but 2 weeks post emergence males tended to have a longer tibia than females (Tab.1). At 4 weeks post emergence and prehibernation this difference was significant (Tab.1).

Foot length was similar in males and females at natal emergence but in the following three phases males had larger feet than females (Tab.1).

Table 1. Comparison of the morphometric parameters (Mean ± SD) in females and males

	Morphometric Parameter									
	Head (mm)					Tibia (mm)				
Phase	Sex	Mean	SD	T Z	P	Mean	SD	T Z	P	N
Natal Emergence	Female	38.45	2.80	0.430	0.668	30.82	2.85	-0.528	0.598	44
	Male	38.07	5.36			31.10	3.40			56
2Weeks Post Emergence	Female	42.50	2.13	-0.445	0.657	34.97	2.32	-1.849	0.065	29
	Male	42.63	1.48			36.50	2.90			33
4Weeks Post Emergence	Female	44.17	1.51	-2.182	0.029	37.18	2.21	-3.951	0.000	21
	Male	45.15	1.83			40.10	2.40			28
Prehibernation	Female	47.51	1.92	-0.968	0.333	40.98	2.23	-3.625	0.000	13
	Male	48.20	1.86			43.33	2.26			27
	Foot(mm)									
Phase	Sex	Mean	SD	T Z	P	N				
Natal Emergence	Female	26.50	2.10	-0.854	0.393	44				
	Male	27.10	2.50			56				
2Weeks Post Emergence	Female	28.86	1.41	0.267	0.000	29				
	Male	30.43	1.41			33				
4Weeks Post Emergence	Female	29.80	0.92	-4.588	0.000	21				
	Male	31.70	1.30			28				
Prehibernation	Female	30.58	0.66	-3.684	0.000	13				
	Male	32.24	1.31			27				

Comparison of early and late born juveniles

Body mass at natal emergence did not differ significantly between early and late born juvenile hamsters but late born juveniles tended to emerge with a higher mass than early born ones (Fig.14). Two weeks later the difference was significant (Fig.15). However, 4 weeks post emergence no difference between the two groups was found (Fig.16), while at prehibernation early-born juveniles were heavier than those born late in the season (Fig.17).

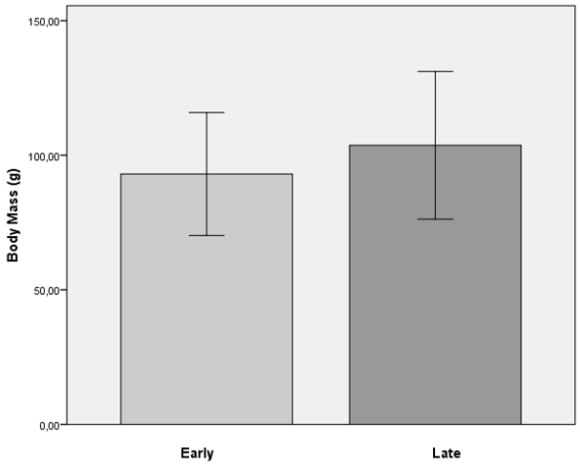

Fig.14. Body mass (g) at natal emergence in early and late born juvenile hamsters (mean ± SD, early born n=81, late born n=19, p=0.057)

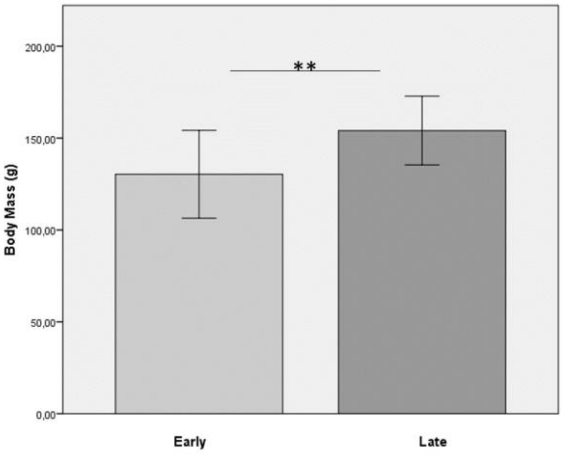

Fig.15. Body mass (g) in early and late born juvenile hamsters 2 weeks post emergence (mean ± SD, early born n=52, late born n=10, p=0.006).

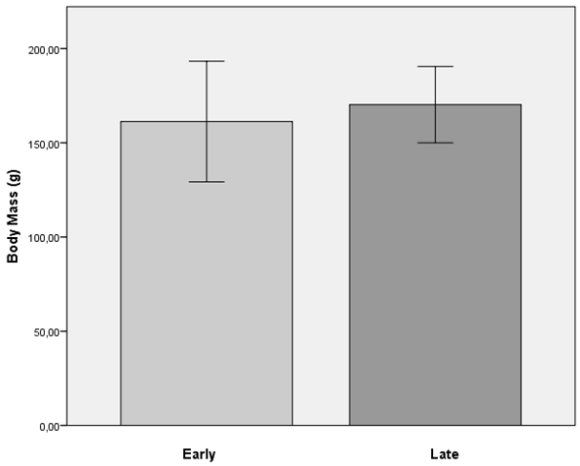

Fig.16. Body mass (g) in early and late born juvenile hamsters 4 weeks post emergence (mean ± SD, early born n=37, late born n=12, p=0.522).

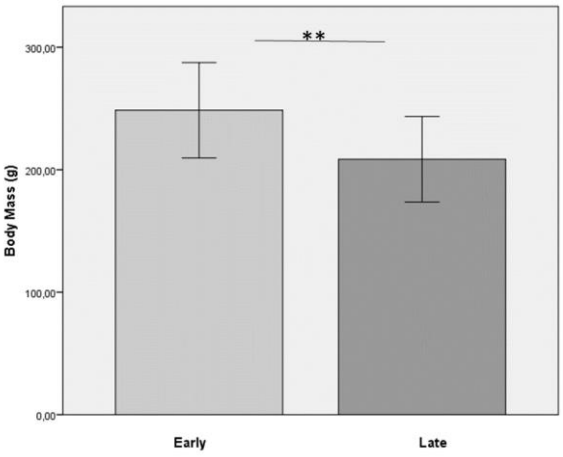

Fig.17. Body mass (g) in early and late born juvenile hamsters at prehibernation (mean ± SD, early born n=29, late born n=11, p=0.007).

The comparison of body fat proportions between early and late born litters revealed no significant differences at natal emergence (Fig. 18) but two weeks later late-born juveniles had higher proportions of body fat than early born ones (Fig. 19). Four weeks post emergence (Fig. 20) and at the end of the active season (Fig. 21) the two groups did not differ significantly in their fat contents.

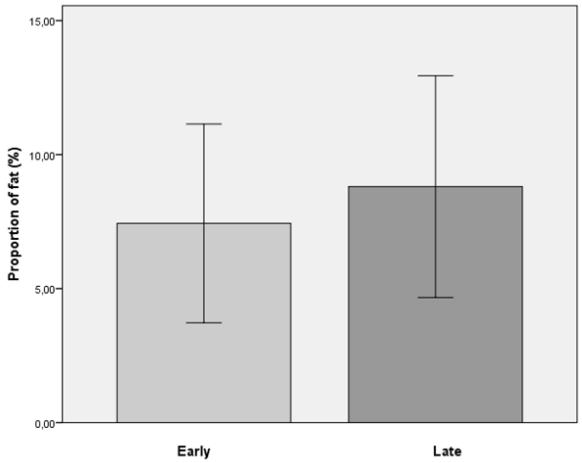

Fig.18.

Proportion of fat (%) at natal emergence in early and late born juvenile hamsters (mean ± SD, early born n=81, late born n=19, p=0.096).

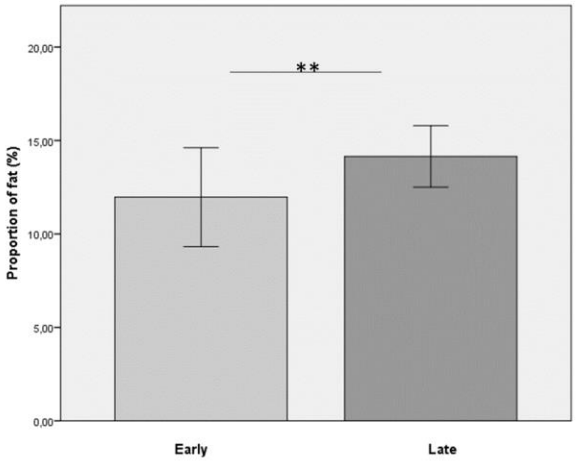

Fig.19.

Proportion of fat (%) in early and late born juvenile hamsters two weeks after emergence (mean ± SD, early born n =52, late born n=10, p=0.006).

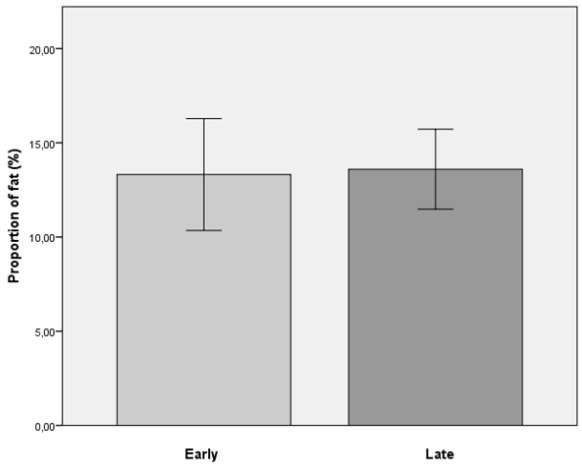

Fig.20.

Proportion of fat (%) in early and late born juvenile hamsters two weeks after emergence (mean ± SD, early born n =37, late born n=12, p=0.365).

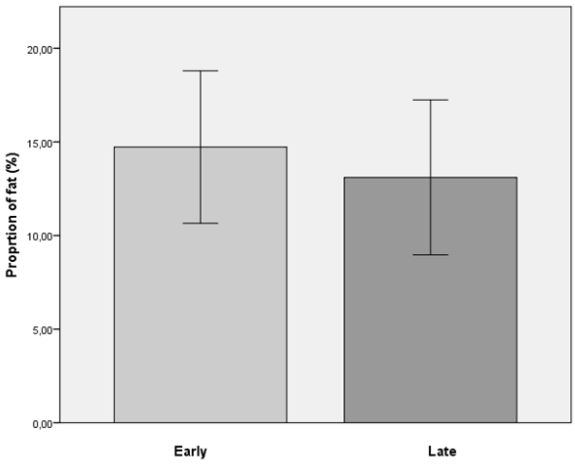

Fig.21.

Proportion of fat (%) at prehibernation in early and late born juvenile hamsters (mean ± SD, early born n=29, late born n=11, p=0.296).

The comparison of the morphometric parameters between early and late born juveniles showed that head length did not differ significantly at vernal emergence as well as 2 and 4 weeks thereafter (Tab.3). However, at prehibernation early born juveniles had a larger head length than late born ones (Tab.3).

Tibia and foot length also did not differ significantly during the first 3 phases. But at prehibernation tibia showed a weak tendency to be larger in early born juveniles (Tab. 3).

Table 3. Comparison of the morphometric parameters (Mean ± SD) of early and late born juveniles, pooled data from 2010 and 2012.

	Morphometric Parameter of both years									
	Head (mm)					Tibia (mm)				
Phase	Birth	Mean	SD	T Z	P	Mean	SD	T Z	P	N
Natal Emergence	Early	38.17	4.65	-0.312	0.756	30.89	3.28	-0.598	0.550	81
	Late	38.52	3.27			31.31	2.65			19
2Weeks Post Emergence	Early	42.45	1.93	-0.862	0.389	35.69	2.91	-0.890	0.373	52
	Late	42.95	0.79			36.25	1.50			10
4Weeks Post Emergence	Early	44.79	1.95	-0.733	0.464	38.80	3.04	-0.326	0.745	37
	Late	44.55	0.90			38.98	1.43			12
Prehibernation	Early	48.35	1.87	-2.440	0.015	43.69	2.85	-1.803	0.071	29
	Late	46.91	1.53			42.08	2.09			11

	Foot(mm)					
Phase	Birth	Mean	SD	T Z	P	N
Natal Emergence	Early	26.74	2.25	-1.301	0.193	81
	Late	27.15	2.59			19
2Weeks Post Emergence	Early	29.54	1.63	-1.777	0.081	52
	Late	30.51	1.24			10
4Weeks Post Emergence	Early	30.84	1.53	-0.198	0.843	37
	Late	30.98	1.42			12
Prehibernation	Early	31.71	1.42	-0.030	0.976	29
	Late	31.66	1.33			11

Early born litters contained up to 12 pups and litter size in late borns ranged from 4-7 pups.

5. Discussion

Juvenile hamsters immerge into their hibernacula after the adult hamsters in October or November, due to the time required to prepare for hibernation consisting of food storage, fat accumulation, structural growth, and finding or building of a burrow (Franceschini & Millesi, 2005; Siutz & Millesi, 2012). Hibernation patterns of juvenile common hamsters varied among individuals. Before and after the hibernation phase, they spend several days to weeks underground at normal body temperatures (Siutz et al. 2016). The accumulated fat reserves as well as food stores could play an important role in the regulation of the time spent in torpor. To get a deeper insight into developmental patterns of juvenile common hamsters, this study aimed to study the course of structural growth versus that of body fat accumulation. To achieve this goal, an appropriate technique to calculate the proportion of body fat in juvenile hamsters in the field had to be used and validated.

Various non-destructive techniques to determine body fat have previously been established including, for example, the **Isotope Dilution Method,** which is based on the fact that fat tissue contains less water than lean tissue. This method uses a certain amount of labelled water (deuterium, tritium and oxygen (^{18}O)) to estimate the water content. In order to estimate body fat content this technique uses the inverse relationship of body water and of body fat (Mata et al., 2006). Isotopes are injected into the living subject and after approximately 90 minutes, tracer and body water are successfully mixed (Eichhorn & Visser, 2008). This method is accurate and ideally rerun to investigate groups of free living and captured animals as well as humans. However, it is expensive and requires a lot of time in the lab (Tidhar & Speakman, 2007; Mata et al., 2015 ;Speakman, 1998).

Total Body Electrical Conductivity (TOBEC) is a method to estimate body composition using the difference in water content between fat and lean tissue of the organisms under investigation. Lean body components have a high content of water and electrolytes and are therefore good conductors. By contrast, fat tissue is nearly anhydrous and thus not a good conductor for electricity (Zuercher et al., 1997; Pethig, 1979). This method is quick, painless, and less stressful for the animals and can be accurate if the subjects are well cared for (not hypothermic, well hydrated, and correctly positioned) (Zuercher et al., 1997; Robin et al., 2002). Despite the technique's advantages it is still time consuming due to the handling of the

subjects and the need to find appropriate adaption plus the high costs (Tidhar & Speakman, 2007).

*"**Dual Energy x-ray absorptiometry (DXA)** uses two x-rays, one of a high energy dose (70-90 KeV) and one of a low energy dose (15-40Kev)"* (Stevenson & van Tets, 2008). However, this method is problematic due to the fact that the animals have to be motionless (which requires anaesthesia) in addition to the various software and hardware, which must be calibrated validated and compatible (Johnston et al., 2005; Secor et al., 2003; Korine et al., 2004). Furthermore, the technique is quite expensive and time consuming.

Morphological measurements is a method which scales different body parts such as the head, tibia and foot and body mass. In order to accomplish such an investigation the specimen are caught, measured, and released. Therefore, it is a quick, cheap and a much more harmless method for the subjects. Another advantage lies in the repeatability making it ideal for the investigations particularly of endangered species (Pluch, 2013; Weissinger, 2013; Tidhar & Speakman, 2007).

Since the first three above mentioned methods are complex, expensive and often require special equipment or a high number of subjects (Tidhar & Speakman, 2007), we applied the latter method, morphological measurements. By using this technique, which has already helped to create a model for adult and sub adult common hamster (Siutz et al., 2012), we were able to explain 96% of the variance of the proportion of fat in juvenile common hamsters.

5.1. Comparison of female and male juveniles

Body mass increases can include both structural growth and fat accumulation. In male juvenile hamsters body mass was significantly higher than in females from four weeks post emergence until pre-hibernation.

The reason for this sexual dimorphism may lie in the preparation for mating in the upcoming year. Males end their hibernation phase earlier than females in order to activate their reproductive system (Eibl-Eibesfeld, 1953; Weinhold & Kayser, 2006). The males emerge from their hibernaculum with elevated androgen levels and with scrotal testes (Barnes et al. 1988; Siutz et al., 2012; Franceschini-Zink & Millesi, 2008). Another reason for the earlier end of their hibernation phase is to regain strength to win fights against other males during intrasexual

competition (Le Galliard et al., 2006), and to mate with the oestrous females in their extended home range during the mating season (Kayser et al., 2003).

Our results showed that the sexual dimorphism in **growth** (head, tibia and food) was already established at 4 weeks after natal emergence and continued until pre-hibernation (Niethammer, 1982; Weinhold & Kayser, 2000; Heimann, 2013). The only exception was head length, which did not differ significantly between the sexes after 4 weeks post emergence. This showed that males invest more in structural growth, which is related to the promiscuous mating system, characterized by high intrasexual competition among males (Stumpfel et al., 2016). Smaller males will not succeed in aggressive interactions against older and bigger males, as suggested by studies of Siutz (2012), (Delville et al., 2003). Thus, males usually start mating as yearlings to have more time to invest in structural growth and have better chances to reproduce successfully (Siutz et al., 2012). Although juvenile hamsters have been observed to mate, it is rather uncommon (Franceschini et al., 2007; Franceschini-Zink & Millesi, 2008; Pluch et al., 2013; Weissinger, 2013; Siutz & Millesi, 2012).

On the other hand, when comparing **body fat**, we found significantly higher fat proportions in juvenile female hamsters during pre-hibernation. This can be explained by the fact that adult females under natural conditions would have to cope with pregnancies and the probability of raising up to 3 litters per year (Franceschini-Zink & Millesi, 2008; Pluch et al., 2013; Andersen et al., 2000). One reason why the juveniles start to fatten up during pre-hibernation might be to contribute to the upcoming active season. They do not only need the fat reserves to survive the winter, but also to be in full strength in case they will mate and have pups (Speakman, 2008). Reproduction in the first season is uncommon but possible (Franceschini et al., 2007; Franceschini-Zink & Millesi, 2008; Pluch et al., 2013; Weissinger, 2013;Siutz & Millesi, 2012) in contrast to garden dormice which can reproduce in their birth year (*Eliomys quercinus*) (Stumpfel et al., 2014).

Our result contrasts the findings of Siutz et al. (2012), in which both juvenile and adult male hamsters had higher fat proportions than females of the same age group. The authors explained these differences based on observations of foraging behaviour, showing that males more frequently feed on the surface while females almost exclusively cache food during the active season (Siutz et al. 2012).The differences of our results may be caused by our pooled data set, maybe some possible difference got revoked through the separate data from Pluch

(2013) and Weissinger (2013). However, this was not confirmed due to a lack of a model to calculate body fat in juveniles.

5.2. Comparison of early and late born juveniles

Late born juveniles already tended to emerge with a higher body mass than early born juvenile hamsters. Two weeks later, this difference in body mass was significant. As a result of their older age however, the early borns where heavier during pre-hibernation.

The late borns are mostly 2ndand 3rd litters or originated from females that reproduced late in the season. Thus lower maternal investment due to previous reproductive effort or perhaps lower energy reserves in young individuals could be assumed. In addition the pups have to cope with a shorter time span after weaning to dig or find an appropriate burrow and food for development and food storage until the end of the season, in contrast to the early born which have more time to prepare for the winter season. As a consequence, mortality rates of late born pups are high, because of the lack of available time to fully compensate energy demands before the winter onset and perhaps higher exposure to predators (Siutz & Millesi, 2012; Weissinger, 2013; Franceschini-Zink & Millesi, 2008;

However, one positive effect of being born late in the season is the small litter size. In our study, early pups were born mostly in a bigger litter size up to 12 in contrast to the late borns with 4-7 pups per litter. This could lead to a higher individual body mass in smaller litters due to a higher fat content of milk. Additionally, the pups do not have to prevail against too many siblings during the lactation period (Franceschini-Zink & Millesi, 2008; Pluch et al., 2013; Heimann, 2013). A higher fat content in mother´s milk could be caused by a decrease of the pressure of the mammary gland lumina in smaller litters (Krebs & Singleton, 1993). So the fatty milk could be "a head start" because of the disadvantage of being born late in the season (Pluch, 2013).

An alternative explanation for our findings could be related to the investigation of Stumpfel et al (2016) who showed that in garden dormice the late borns gained fat much faster than the early borns to improve the chance of overwinter survival even though they cannot fully compensate for the shorter time to prepare for the winter onset like the early born. The author referred to Körtner and Geiser (1995), which supposed increased food intake or/and reduced activity as factors for the outcome. Both results are in line with the outcomes from Bunnel

(2009) who worked with European hedgehogs (*Erinaceus europaeus*) and reported that late borns gained body mass faster than early borns. He assumed that shorter day length combined with a falling of ambient temperature leads to altering in the physiology of the hedgehog like an increase in appetite and better usage of the food, therefore an increase body mass.

There were no significant differences in **structural growth** (head, tibia and foot) which is in contrast to the findings of Stumpfel et al (2016) showing that the late born garden dormice had higher structural growth rates at post weaning. The reason for our findings could lie in the combined data from Weissinger (2013) and Pluch (2013). Weissinger also found in her investigation no significant differences in head, tibia and foot like in our pooled data set. Pluch (2013) on the other side found differences in the first and second litters. So it can be assumed that there was a possible effect in the pooled data.

Both investigations did not mention any environmental disturbance, which could have an effect on finding enough food resources. Weissinger (2013) assumed that the low reproductive outcome in the investigated year could be an issue for the results. The females may invest more in their litters for example when they gave birth late in the season. This could be the reason, that these late litters were cared for long enough to diminish the possible differences in structural growth.

Our results showed that the higher body mass and higher proportion of fat of the late borns could be an adaptive mechanism, because of the shorter time to prepare for hibernation.

Summarizing we can say, that based on the model we were able to gain insight developmental patters in common hamsters without disturbing and stressing the animals. This non-invasive method, may help to get a better insight in sex differences and critical phases during juvenile development particularly in offspring born late in the season.

6. References

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7. Appendix

7.1 Zusammenfassung

Tiere, die in saisonalen Öksystemen beheimatet sind, müssen mit den knapp werdenden Ressourcen in der Winterzeit und der damit verbundenen niedrigen Temperatur umgehen können. Einige Tierarten bewerkstelligen dies mit Migration, geringerer Aktivität oder Winterschlaf (Hibernation). Feldhamster (*Cricetus cricetus*) sind fakultative Winterschläfer, welche eine Kombination aus Sammeln von Vorräten und Körperfettzuwachs nutzen um Reserven für die Winterperiode anzulegen.

Eine Besonderheit der Feldhamster ist ihre für Winterschläfer relativ hohe Reproduktionsrate. Weibchen können bis zu drei Würfe pro Saison haben. Die Überlebensrate der Jungtiere hängt nicht nur von der mütterlichen Konstitution und Fürsorge ab, sondern auch vom Zeitpunkt der Geburt in der sechsmonatigen aktiven Phase. Bis zum Herbst müssen die Jungtiere wachsen und Fett-Speicher-Depots anlegen. Zudem muss ein geeigneter Bau gefunden oder gegraben werden, um Vorratskammern anzulegen.

Das Ziel der Untersuchung war die Weiterentwicklung einer Methode auf nicht-invasive Weise anhand von morphometrischen Parametern wie Körpergewicht, Kopf- Tibia und Fusslänge den prozentuellen Fettanteil zu ermitteln. Die bisherige Methode für Jungtiere in den ersten Wochen nicht geeignet. Daher wurde von Kadavern mithilfe der Soxhlet-Apparatur der Fettgehalt bestimmt und anhand der morphometrischen Parameter ein statistisches Modell entwickelt, das die Kalkulation des Fettgehalts ermöglichte. Im zweiten Teil der Arbeit wurde Gewichtsveränderungen und strukturelles Wachstum und der Körperfettanteil bei männlichen und weiblichen Jungtieren sowie früh und spät im Jahr geborenen Jungen zu 4 Zeitpunkten verglichen. Dafür wurden in das erstmalige Auftauchen aus dem Bau nach der Geburt, zwei und vier Wochen danach sowie kurz Beendigung der Aktivität außerhalb des Baus im Herbst ausgewählt.

Ein Geschlechtsdimorphismus entwickelte sich bei den Jungtieren vier Wochen nach dem erstmaligen Auftauchen aus dem Bau. Männchen zeigten ab dieser Phase ein höheres Körpergewicht als Weibchen, das, wie sich in den Längenmaßen zeigte, in erster Linie auf schnelleres strukturelles Wachstum zurückzuführen war. Im Körperfettgehalt zeigte sich in dieser Phase kein Unterschied. Kurz vor Winterbeginn war bei den Weibchen ein höherer Fettwert erkennbar als bei Männchen. Die ausgeprägtere Wachstumsrate bei Männchen ist vermutlich auf die hohe intrasexueller Konkurrenz der Jährlingsmännchen in der Paarungszeit

ausgesetzt sind zurückzuführen. Nur Männchen die in guter Kondition und mit entsprechender Größe aus den Winterschlafbauten auftauchen haben Chancen erfolgreich zu reproduzieren. Spät geborene Jungtiere wiesen wegen des höheren Fettanteils zwei Wochen nach dem erstmaligen Auftauchen aus dem Bau ein höheres Gewicht auf als früh im Jahr geborene Junge. Das lässt sich mit der geringeren Wurfgröße erklären, durch welche die Tiere mehr Zugang zur fettreichen Muttermilch hatten. Vor Winterschlafbeginn waren aufgrund ihres höheren Alters die Früh-Geborenen in Gewicht und Körpergröße überlegen. Der relative Fettanteil wies keine signifikanten Unterschiede auf, womit gezeigt werden konnte, dass die früh geborenen Jungen den Zeitvorteil in strukturelles Wachstum investiert hatten. Dies könnte für die Fortpflanzung in der folgenden Saison von entscheidender Bedeutung sein. Für die spät geborenen jungen Hamster stand vermutlich das Überleben der Winterperiode im Vordergrund.