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Final dissertation project

Análise da dieta de pequenos mamíferos: un traballo experimental con *Micromys minutus*

Análisis de dieta de pequeños mamíferos: un trabajo experimental con *Micromys minutus*

Diet analysis of small mammals: an experimental study with *Micromys minutus*

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ABSTRACT

The ongoing growth of the human population and human activities have caused a rapid loss of biodiversity. Species play an important role in the ecosystem functioning. Thus, in order to conserve them, detailed insight into their biology is crucial. The Harvest mouse (*Micromys minutus*) is an understudied species whose populations across the UK have undergone an apparent decline of the 71 % over the past 18 years. Furthermore, there seems to be little information available on the diet of this species, which can be essential to improve welfare guides for captive populations as well as for future conservation actions for wild populations. Thereby, the aim of the present study was to shed some light on the diet of a captive population of *Micromys minutus* at the Wildwood Trust by assessing their (1) dietary preferences, (2) potential sex differences, (3) feeding time patterns and (4) intake rates. In order to do so, 5 individuals of Harvest mice were presented with different feeds and as a result their consumptions over a period of 14 days were obtained. Posteriorly, data was analysed by means of variance analysis (ANOVA) performed with SPSS, showing significant differences in the food intake of *Micromys minutus* depending upon food type and feeding time effects. The study revealed that the mice preferred blackberries, canary seeds, dried meal worms, naked oats and safflowers over white and red millet, although males had a higher consumption of dried meal worms and naked oats, whilst females preferred safflowers. Moreover, mice showed greater nocturnal activity as well as a daily consumption of more than their 30% of their body weight in food.

Key words: Harvest mouse, *Micromys minutus*, captive populations, Wildwood Trust, Diet, Food preferences, Intake.

RESUMO

O continuo crecemento da poboación humana e as súas actividades causaron unha rápida perda de biodiversidade. As especies desempeñan un papel importante no bo funcionamento do ecosistema. Polo tanto, para preservalos, unha visión detallada da súa bioloxía é crucial. O rato espigueiro (*Micromys minutus*) é unha especie pouco coñecida cuxas poboacións no Reino Unido experimentaron un descenso aparente do 71% nos últimos 18 anos. Ademais, parece haber pouca información dispoñible sobre a dieta desta especie, a cal pode ser esencial para mellorar as directrices benestar para as poboacións en cativerio, así como para futuros esforzos de conservación para as poboacións salvaxes. Polo tanto, o obxectivo deste estudo foi aportar aclaracións sobre a dieta dunha poboación cativa de *Micromys minutus* no Wildwood Trust avaliando as súas (1) preferencias alimentarias, (2) posibles diferenzas sexuais, (3) patróns nas horas de alimentación e (4) inxestas. Para iso, 5 individuos de rato espigueiro recibiron diferentes alimentos e como resultado, obtívose o seu consumo durante un período de 14 días. Posteriormente, os datos foron analizados por análise de varianza (ANOVA) utilizando o programa SPSS, que mostrou diferenzas significativas na inxestión de alimentos de *Micromys minutus* con respeto ós tipos de comida e á hora de alimentación. O estudo revelou que os ratos preferiron moras, alpiste, vermes secos, avea e cártamo ó millo branco e vermello, aínda que os machos tiveron un maior consumo de vermes secos e avea, mentres que as femias preferiron o cártamo. Ademais, os ratos demostraron maior actividade nocturna, así como un consumo diario de alimento de máis do 30% do seu peso corporal.

Palabras clave: Rato espigueiro, *Micromys minutus*, poblacións cativas, Wildwood Trust, Dieta, preferencias alimenticias, inxesta.

RESUMEN

El continuo crecimiento de la población humana y sus actividades ha causado una rápida pérdida de biodiversidad. Las especies juegan un papel importante en correcto funcionamiento del ecosistema. Por lo tanto, para conservarlas, una visión detallada de su biología es crucial. El ratón espiguero (*Micromys minutus*) es una especie poco estudiada cuyas poblaciones en el Reino Unido han experimentado una disminución aparente del 71% en los últimos 18 años. Además, parece haber poca información disponible sobre la dieta de esta especie, la cual puede ser esencial para mejorar las guías de bienestar para las poblaciones cautivas, así como para futuras acciones de conservación para las poblaciones silvestres. Por lo tanto, el objetivo del presente estudio fue arrojar algo de luz sobre la dieta de una población cautiva de *Micromys minutus* en el Wildwood Trust mediante la evaluación de sus (1) preferencias dietéticas, (2) posibles diferencias sexuales, (3) patrones en el horario de alimentación y (4) ingesta. Para ello, 5 individuos de ratón espiguero recibieron diferentes alimentos y como resultado se obtuvieron sus consumos durante un período de 14 días. Posteriormente, los datos se analizaron mediante análisis de varianza (ANOVA) mediante SPSS, que mostró diferencias significativas en la ingesta de comida *Micromys minutus* según los factores tipo de alimento y hora de alimentación. El estudio reveló que los ratones preferían las moras, el alpiste, los gusanos secos, la avena y el cártamo sobre el mijo blanco y rojo, aunque los machos presentaron un mayor consumo de gusanos secos y avena, mientras que las hembras prefirieron el cártamo. Por otra parte, los ratones mostraron una mayor actividad nocturna, así como un consumo diario de alimento de más del 30% de su peso corporal.

Palabras clave: Ratón espiguero, *Micromys minutus*, poblaciones cautivas, Wildwood Trust, Dieta, preferencias alimenticias, ingesta.

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1. Introduction

The ongoing growth of the human population and human activities have resulted in a rapid loss of biodiversity. Species play an important role in the ecosystem functioning and ultimately to goods and services that humans benefit from. Thus, the precautionary principle should probably be applied to development and conservation decisions so as to continue with the existence of species (Gascon et al., 2015). However, detailed insight and understanding into the biology of the species is key to conserving them and to raising awareness among society.

The Harvest mouse (*Micromis minutus*) is a species that has barely been studied, as according to most authors (Dickman 1986, Harris et al. 1995, Rudd 1998 & Nores 2012) reading material on the subject of harvest mouse seems rather scanty. This is probably because the species causes no economic damage, nor does it present any interesting features from a scientific point of view. On top of that, it is rather difficult to catch. Therefore, there is very limited information on its demographics, ecological characteristics or behaviour. Moreover, studies carried out in Spain have barely contributed towards more data about their biology other than what was previously known about their distribution and habitat information and most of the available work on its biology has been done in Great Britain (Nores, 2012). For the latter reason and owing to the fact that the present study was actually carried out in the United Kingdom, it is mainly focused on the Harvest mice of Great Britain.

1.1. Habitat, distribution and origin

The distribution range of the Harvest mouse includes a wide variety of habitats such as the following: alpine grasslands, tall grass fields, bamboo stands, wetlands, reedbeds, and clearings and edges of humid forest. Moreover, the species seems to be quite adaptable and not dependent upon any particular plant species (Meek, 2011), so it has also managed to thrive in anthropogenic habitats, including: gardens, arable land, drainage ditches, and grain or rice paddies (Aplin et al., 2016). However, their habitat distribution may change depending on the season. Thus, in Summer they appear to dwell in stalk-zone existence, meaning grassy lands with tall vegetation that provide suitable habitats (reed beds, hedgerows, road side verges, gardens, cereal sown fields and any area of rough grassland), while during Winter they adopt a ground-dwelling lifestyle so as to avoid cold conditions; taking advantage of the holes built by other small mammals (Oxfordshire Mammal Group, 2018).

Aplin et al. (2016), describe the current global geographic range of *M.minutus* to include mainly the Palearctic and Indomalayan regions (Fig. 1), occurring from northern Spain and Great Britain through Europe, eastern Fennoscandia, and Russia to northern Mongolia, China, the Korean peninsula, northeast India, Myanmar, Vietnam, Japan and Taiwan.

In Europe it is present in all areas of the Atlantic and Central and Eastern Europe. Nevertheless, it is absent from Ireland, most of the Scandinavian

Peninsulas (only present on the border between Sweden and south-east Norway), Italic Peninsulas, Balkan Peninsulas and completely absent in the Mediterranean islands. In Spain the species can be found mainly in the North; concretely along the Cantabrian region that extends eastwards from the centre of the Asturias and throughout the area of Navarra (Nores, 2012). In the UK, the distribution of the harvest mouse ranges from central Yorkshire to southern England and some parts of coastal Wales (Fig. 2). Other colonies outside this area probably have to do with accidental introductions (Harris et al., 1995).

Obtaining paleontological or archaeological evidence of the Harvest mouse's presence is not a straightforward task because of both its size and the particularities of its habitat, scarce in the Pleistocene and much of the Holocene, so the history of its colonization is not very well known. The genus appeared in Europe at the end of the Pliocene as an element of steppe fauna. Suitable habitat and adequate conditions for the habitat expansion of *M.minutus* arose after the ice removal, the clearance of woodlands and the increase of croplands and grasslands (Nores, 2012). Nonetheless, how much of this spread was due to natural colonization as opposed to further accidental translocations seems to be still unknown, as some authors consider *M.minutus* to be a native species, whereas others believe it to be an accidental post-glacial/Neolithic introduction to Western Europe (Harris 1979 & Harris et al.1995).

Figure 1. Geographic range of *M.minutus*. Source: IUCN Red List of Threatened Species.

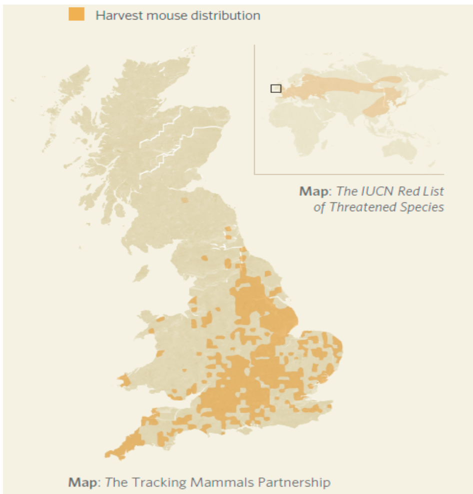

Figure 2. Distribution range of *M.minutus* in the UK. Source: The Tracking Mammals partnership.

1.2. Density and conservation status

Density estimates are particularly difficult to calculate for Harvest mouse, as it is hard to trap and often not detected even when present (Aplin et al., 2016). The most effective methods to study Harvest mice populations appear to be the analysis of Barn owl pellets and nest searching (Meek, 2011). The species is limited in its distribution, but locally can occur in large numbers. The population estimate for the UK is about 1 415 000 individuals in England and 10 000 in Wales (Battersby and Tracking Mammals Partnership, 2005). Furthermore, population density varies with habitat; being the highest in reedbeds (20-50/ha) and the lowest in cereal fields (0.05-0.4/ha) (Forder, 2006).

The IUCN (International Union for Conservation of Nature) has categorised the Harvest Mouse as Least Concern (LC) (Aplin et al., 2016), which describes a "species that has been evaluated against the criteria and do es not qualify for Critically Endangered, Endangered, Vulnerable or Near Threatened. Widespread and abundant taxa are included in this category" (IUCN, 2001). This is justified since it appears to be that "the species has a very wide distribution, is common and adaptable, and faces no major threats" (Aplin et al., 2016). Previous assessments include Least Concern in 2008 and Lower Risk/Near Threatened in 1996. However, certain criteria used by the IUCN based on the area of distribution has been proved inefficient to describe local and regional populations, as it just takes into account the global conditions of a species in order to assess their category, without considering their regional conditions. Thus, population declines have been reported in several parts of Europe (Aplin et al., 2016).

In the UK numbers are thought to be declining. Over the last century major fluctuations in the numbers of the British populations were noted, which led to conduct national surveys. In 1973 the first detailed investigation was commissioned by the Mammal Society, whose aim was to establish the status of the Harvest mouse in Britain, determine its true distribution and collect data on the habitat requirements of the Harvest mouse (Meek 2011 & Harris 1779). This survey was repeated in 1996 and 1997, showing an apparent decline of the 71% over the past 18 years (Oxfordshire Mammal Group, 2018), in contrast to studies from authors such as Harris (1779), which suggest that there is no evidence for a decline and attribute it to "a lack of competent recorders" (Harris, 1779).

Despite the fact that populations of Harvest mouse fluctuate drastically and that some reported declines may have had to do with natural fluctuation (Aplin et al., 2016), possible reasons for the huge decline are thought to be involved with the habitat loss caused by the agricultural practises as well as with climate change. Therefore, main threats include: reduction in hedgerows due to modern farming methods, ripening of crops coincides with breeding season, combine harvesting, stubble burning, pesticide use and flooding of reedbeds (Battersby and Tracking Mammals Partnership 2005 & Forder 2006).

Not only is the Harvest Mouse less widespread than previously thought, but severely threatened in its current range within the UK. Nevertheless, in order to reverse this populations decline, since 2007 *M.minutus* is a UK BAP Priority Species, which includes those species identified as being the most threatened and requiring of conservation action under the UK Biodiversity Action Plan (UK BAP) (Oxfordshire Mammal Group, 2018). It is also included in some Local Biodiversity Action Plans and protected by the Wild Mammals Protection Act,

1996. These actions have been supported with several reintroductions carried out (in 2002, 2003 and 2004) in collaboration with the North of England Zoological Society (NEZS), the Cheshire Wildlife Trust (CWT), the British Association of Shooting and Conservation (BASC) and Chester Zoo (Forder, 2006). Moreover, studies conducted by authors such as M. Meek (2011) have proved the habitat quality and connectivity to be crucial for the persistence of populations of breeding harvest mice and for increasing their numbers.

1.3. Ecology

Harvest mice are extremely active climbers and they may be active at both day and night, though they are particularly so around dusk and dawn. But they seem to show a slight preference for nocturnal behaviour during summer and more of diurnal movement in winter. Despite the fact that harvest mice appear to be predominantly solitary, there is considerable overlap of adjacent home ranges (Forder, 2006). Males can have a territory of up to 400 square metres while female territories are smaller (Wildwood Trust, 2018a).

Mating season is between May and October, but it can last until December depending on weather. They can breed before they are 1 year old, producing in between 3 and 7 litters a year with in between 4 and 8 mice each. They gestation period is 17 to 19 days and the weight of a pregnant female adds up to 15 g before giving birth. New born mice weigh 0.7 to 1 g, being comparatively large animals since they are roughly a 10% of the weight of the female. They are born completely blind and hairless and their tail is not prehensile yet. In spite of their immaturity at birth, they have a short lactation period, so it is highly likely that they start to explore outside the nest quite soon along with developing the ability to climb, being able to climb up a vertical stem at the 10 days old. They then become independent at 15 or 16 years old (Oxfordshire Mammal Group, 2018).

The most obvious sign indicating the presence of *Micromys minutus* are its nests (Fig. 3), they are the only British mammals that build ball-shape nests of woven grass. The diameter of the nests is 7-12 cm and they seem to lack a definite entrance hole. Nests are mainly made out of monocotyledonous plants and tend to be found in dense vegetation such as grasses, rushes, cereals, grassy hedgerows, ditches and brambles (The Mammal Society, 2018). Harvest mice build two different types of nests depending on whether it is breeding season or not. Non-breeding nests are smaller (around 5 cm diameter) and they are built very quickly by both males and females throughout the year and they are usually built closer to the ground, providing the mice with temporary shelter from predation and bad weather. In contrast, breeding nests are bigger (10-12 cm diameter) and they seem to be much more elaborate than non-breeding nests as they take longer to build (up to 7 days depending on weather conditions). Additionally, breeding nests are usually built higher above the ground (at around 30 cm). A pregnant female harvest mouse starts building her nest 10 days before giving birth. The structure of these nests is essential for thermoregulation, as if it gets soaked, it will increase the heat loss (Oxfordshire Mammal Group 2018 & The Mammal Society 2018).

The life span of the Harvest mice in the wild is 18 months on average, but they are often unlikely to make it up to more than 6 months. In captivity they can live up to 4 years (Forder, 2006). Cold weather is a major cause of mortality; hence Britain's heavy rain and sudden frosts during the autumn and winter seasons can kill around 95% of the population, including 80% of the late-born litters of mice before they even leave the nest (Oxfordshire Mammal Group, 2018). Apart from that, they have many predators. Not only are they predated by most generalist predators of small mammals, such as medium and small carnivores (like cats, weasels, stoats and foxes) and raptor birds (like owls and hawks), but they are also predated by other animals not specialised in the predation of vertebrates such as rats, corvids, shrikes and even pheasants, which capture especially young individuals (Nores, 2012).

Figure 3. *M.minutus* nest. Source: Worcestershire Mammal Group.

1.4. Diet and energy requirements

Little seems known about natural diet of harvest mice in the wild; "There are no quantitative data on the diet of harvest mice in Britain" (Harris et al., 1995). On the whole, they are a truly omnivorous species and their diet consists predominantly of a mixture seeds (grass, millet, oats and wheat), fruits, berries and grain from cereal heads, leaving characteristic sickle-shaped remains. However, hardly ever is any damage to cereal crops noticed (The Mammal Society, 2018). In addition, they can also feed on insects (moths, caterpillars, flies and grasshoppers), flowers, moss, roots, fungi, meat and even bird eggs (Aplin et al., 2016).

In captivity there appear to be different opinions. As mice, they can feed on a variety of preparations such as mice and rabbit food and bird food. Their basic diet normally consists of mixed millet ('quail mix', 'special budgie' or 'aviary mix'), as seeds seem to be preferred. This diet can be complemented with a variety of grasses, mosses and blackberries. Additionally, they can sometimes be offered fruit and vegetables, such as apples and carrots, and some sort of animal protein, like mealworms and dog food. Regarding water needs, they seem to drink very little due to their tiny size, but they dehydrated very quickly (Rudd, 1998).

According to research, Dickman (1986) seems to be the only author who has conducted a study to provide information about the diet of the Harvest mice. The study consisted in describing the distribution of *Micromis minutus* in habitat patches in the city of Oxford along with providing a preliminary assessment of the diet of those urban populations. The latter was assessed by means of faecal analysis, collecting pellets from used traps or from within the breeding nests and then classifying foods into broad dietary categories. The results obtained showed that *M. minutus* was omnivorous in each habitat, with seeds, fruit, green leaves and insects being consistently well represented, whereas other invertebrates (mainly spiders and snails), fungus, moss, vertebrate and root material appeared less frequently. They also showed that the species is clearly omnivorous in each habitat, being seed the major dietary component in long grass and scrub, but fruit predominated in orchards due to the local abundance of apples and blackberries. Consumption of monocotyledon and dicotyledon leaves was highest in long grass and scrub, and again due to local abundance. Furthermore, the invertebrate remains were mainly adult and larval beetles (families Scarabaeidae, Chrysomelidae and Coccinellidae), lepidopteran larvae (family Noctuidae) and small homopteran bugs. In short, the study found that in broad terms the main components of the diet of Harvest mice in Oxford consisted of seeds (45%), leaves (25%), insects (15%) and fruits (6%) (Fig. 4).

Turning to the second point, Harvest mice have high energy requirements as they are endotherms and have to cope with a high surface to volume ratio because of their small size (Forder, 2006). Apparently, seeds are the most important item in their diet because of their high energy content. When living on seeds, Harvest mice consume about 30% of their own weight each day (Fig. 5) (The Mammal Society, 2018).

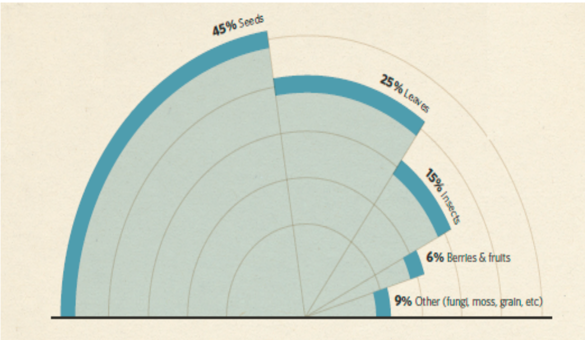

Figure 4. Composition of *M.minutus* diet, resulted from the study carried out by Dickman in Oxford. Source: Oxfordshire Mammal Group.

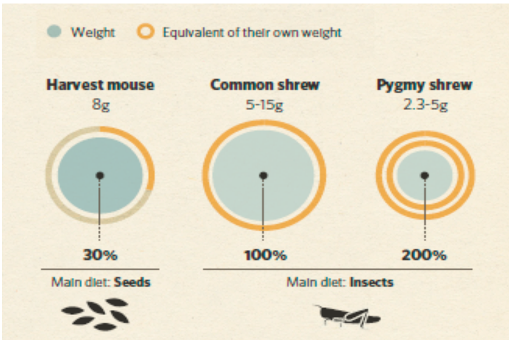

Figure 5. Energy requirements of *M.minutus* in comparison to that of the Common shrew and the Pygmy shrew. Source: Oxfordshire Mammal Group.

2. Objectives

In view of the general lack of information on *Micromys minutus*, and especially due to the current situation with the apparent declines in their populations across the UK, nowadays there appears to be an urgent need to do research and gain further insight into the biology of this species. As not only may research be helpful to improve guides for the welfare of captive populations, but also for future conservation actions of wild populations.

Since apparently there is just one previous study assessing the diet of Harvest mice, the aim of this project is to shed some light on the following aspects of the diet of captive *Micromys minutus*: (1) Dietary preferences, (2) potential differences in between males and females, (3) feeding time patterns and (4) intake rates.

3. Material and methods

3.1. Study species

The Harvest mouse (*Micromis minutus*) (Fig. 6) was first described in the UK by the naturalist Gilbert White in Hampshire in 1767 (Meek, 2011), it belongs to the family Muridae and the order Rodentia, being the smallest rodent in Europe and one of the smallest mammals in the world. The genus Micromys is monospecific and is remotely related to the Apodemus-Mus group, having separated from this line in the Pliocene (about 7 million years ago), although there seems to be no complete agreement on whether all European populations are monotypic or if there is a particular subspecies in southern Europe (Nores, 2012).

M.minutus can be easily distinguished from other species of mice just by their particularly tiny size, as the head and body length ranges from 50 to 80 mm, weighing 6g on average when fully grown. They are the only British mammals with a truly prehensile tail for climbing, being almost the same length as the head and the body (50-70 mm). Their face rounded, with a blunt nose and small, round and black eyes (about 3 mm diameter). Their ears are rather small, rounded and hairy, with large resonating chambers, which are believed to enable the mouse to detect low frequency sounds carried over great distances, and therefore better able to escape predation. The feet are fairly broad with the outer of the five toes on each foot being large and more-or-less opposable as they are specially adapted for climbing. These mice can grip a stem with each hindfoot and its tail, leaving the forepaws free for collecting food. Furthermore, the fur of the Harvest mice is quite distinctive; its back is russet in colour and its belly is white and fairly demarcated from the flanks (Oxfordshire Mammal Group, 2018).

Figure 6. Harvest mouse (*Micromis minutus*). Source: Paul Tymon Photography. Harvest Mice Workshop.

3.2. Study site

The Wildwood Trust is a wildlife park and also a registered Charity located in the county of Kent (England, United Kingdom), whose aim is to save British Wildlife from extinction and reintroduce recently made extinct animals. Many of the animals that can be seen there are now either extinct in the UK or are under threat and as a conservation charity, Wildwood is dedicated to protecting Britain's most threatened wildlife.

In 1997 Cheshire Wildlife Trust and Chester zoo began a Harvest mouse breeding project to provide animals for reintroduction across Cheshire and the Wildwood Trust conservation team joined the project in 2003 (Thomasz et al., 2017), using old tennis balls from Wimbledon with an entrance hole drilled in the side as a novel way of reintroducing harvest mice to the wild (Wildwood Trust, 2018b). Furthermore, the Wildwood Trust is working in collaboration with the Kent Mammal Group on a 5-year project to find out the current distribution of species across the county of Kent. Such survey has not been undertaken in Kent before, and most of the old records are anecdotal. The aim of the survey is to create a presence/absence map for each 4km squared grid space across the whole county by searching Harvest mice nests. The project is entering its fourth season, which begins in September 2018 (Wildwood Trust, 2018c).

The conservation team of Wildwood operates in an area restricted to the public and although its main focus are the captive breeding and reintroduction programmes of the water vole (*Arvicola amphibius*), it is also in charge of other small mammals, including Harvest mice (*Micromys minutus*). Out of the current stock of Harvest mice at Wildwood, some were bred there for reintroduction purposes, while others were rescued and brought there. They are not on display and they are currently used by the education team either to give awareness talks to public and schools or for scientific research.

The present project took place in the rodent room located within the conservation yard at the Wildwood Trust.

3.3. Study mice and feeds

From the Harvest mice of the rodent room 5 individuals were selected to take part in the project, being 4 males and 1 female.

The mice were contained in their habitual individual glass tank, which differed in size (approximately 60 x 30 x 40 cm). The tanks were covered by a lid with a 0.6 square cm size mesh with a stone on top. The bottom of the tank was covered with wood shavings and quite a substantial amount of hay right on top for the mice to build their nests from along with some leaves and grass. Additionally, it may have also contained a few branches for them to climb and small tubes to provide cover as well as water for them to drink (Fig. 7).

With the purpose of assessing the diet of the Harvest mice, each of them was given a tray with 7 food containers for the different food items in it. These trays were placed at the bottom-front of their tanks (Fig. 7 and 8). The trays had been made out of the edges of card boxes, which were wrapped with black tape in order to refrain the mice from gnawing and/or wetting them, while the food containers used to place the different feeds were actually lids that had been removed from milk bottles.

The number of feeds was decided to be 7 because of the size of the tanks and the lids that could fit in the trays. At the same time, the different food items were selected based on the experience of Wildwood staff and on the actual food mixes and treatments that the mice had been feeding on until that point in time. Apart from that, their accessibility and ease to be tested were also decisive factors in the selection process. Thus, the final 7 food items selected for this project were: safflowers (*Carthamus tinctorius)*, white millet (*Panicum miliaceum)*, canary seeds (*Phalaris canariensis*), red millet (*Panicum miliaceum*), blackberries (*Rubus fruticosus*), dried meal worms (*Tenebrio molitor*) and naked oats (*Avena nuda*) (Fig. 8).

Figure 7. Harvest mouse individual tank of the rodent room at Wildwood with a tray inside. Source: picture taken by me.

Figure 8. Mice feeds selected for the project. Source: picture taken by me.

3.4. Data collection

Prior to the project, weight, sex, age, mouse ID and tank number were recorded as well as the weight of the milk lids used as food containers. Posteriorly, there was an initial pilot period to figure out the most convenient food types and overcome any difficulties that may have arisen through the project. After that, the actual project took place and lasted for 14 days: every day from Sunday to Friday, starting on the 31st of July 2017 and finishing on the 18th of August 2017.

The project consisted of 14 sets of data recording of 24-hour-ish periods, these being split into 2 sets of data recording: (1) a day time period (AM) that would last from 8 a.m. to 4 p.m. and (2) an evening and night time period (PM) from 4:30 p.m. to 8 a.m. of the day after.

First of all, at 8 a.m. in the morning, approximately 2 grams of every feed were weighted with a 3 decimals scale and put into the correspondent lid for every tray (Fig. 9), which were then placed into the correspondent tank for the Harvest mice to feed on (Fig. 10). The food was left there until 4 p.m. in the afternoon, when the trays were removed from the tanks.

Before proceeding with the recordings, as Harvest mice can be quite messy when eating, some food leftovers needed to be picked up from the trays by using tweezers and then placed back into their correspondent lids. Right after that, lids were removed from the trays and weighed with what was left inside. However, in order to obtain the actual value of the intakes, the weight of the lids (which was previously recorded) needed to be subtracted. That value was transferred into an Excel file. Once all the values were obtained, lids containing whichever amount of food was left, were placed back into the trays and these again into their correspondent tanks at 4:30 p.m. So, the remaining food was left from 4:30 p.m. until 8 a.m. in the day after, when trays were again removed from the tanks and the procedure previously explained was applied to obtain the values of the intakes overnight.

Finally, any food leftovers were emptied from the lids and trays and these were then cleaned up so as to be filled up again with the same amounts of fresh food and start a new 24-hour set (Fig. 9). The position of trays was alternated every day in order to avoid biased data, as some food items could have been more accessible than others.

Eventually, an excel file full of data was obtained. This contained the intake during the day (AM) and the intake during evening and night (PM) of the 5 individuals for every food item, as well as the total 14-day consumption. In addition, times when trays were placed into and removed from the tanks along with the temperatures of the room and the position of the trays were also recorded.

Figure 9. Trays containing 2 g of every food type treatment ready to be placed into the tanks. Source: picture taken by me.

Figure 10. Harvest mouse feeding on canary seeds. Source: picture taken by me.

3.5. Data analysis

Experimental design consisted in two crossed factors: “food type” (with safflowers, white millet, canary seeds, red millet, black berries, dry meal worms and naked oats) and “feeding time” (with AM and PM). We analysed differences in food intake by two-way analysis of variance (ANOVA) with “food type” and “feeding time” as fixed effects. When differences were significantly we applied the LSD post-hoc test. Significance level was set at $P < 0.05$. Statistical tests were performed with SPSS Statistics v.24.0 (IBM Corp., Armonk, NY, USA).

4. Results

Our results showed significant differences in the food intake of *M.minutus* depending upon the food type (P-value = 0.042) and on the feeding time (P-value < 0.001). Nevertheless, the captive individuals of *M.minutus* assessed in this study did not show differences in its food intake when it came to analyse the food type preference in regards to the feeding time (food type x feeding time effect: P-value = 0.555) (see Table 1).

Table 1. Results of two-way analysis of variance (ANOVA) to examine the effects of food type and feeding time on intake. Values of P < 0.05 are in bold. See Fig. 9, 12 and 13 for data.

	Intake		
Effect	*d.f.*	*F*	*P*
Food type	6	2.356	**0.042**
Feeding time	1	17.323	**<0.001**
Food type x feeding time	6	0.825	0.555
Error	56		

It can be observed that when having full accessibility to different types of food, the individuals of captive *M.minutus* that took part in the present study had a clear preference for blackberries (mean value= 4.457 g) followed by canary seeds (4.191), dried meal worms (4.087) and naked oats (3.734). On the other hand, safflowers appeared to be fairly consumed (2.739) while their least favourite food treatments seem to be the millets; red (1.061) and white (0.770). Thus, preferred feeds (Blackberries, canary seeds, meal worms and naked oats) showed significant differences with the least consumed feeds (white and red millet), although there seemed to be similarities in between red millet and naked oats. Moreover, safflowers showed no significant differences with the most consumed foods nor with the least consumed (see Fig. 9).

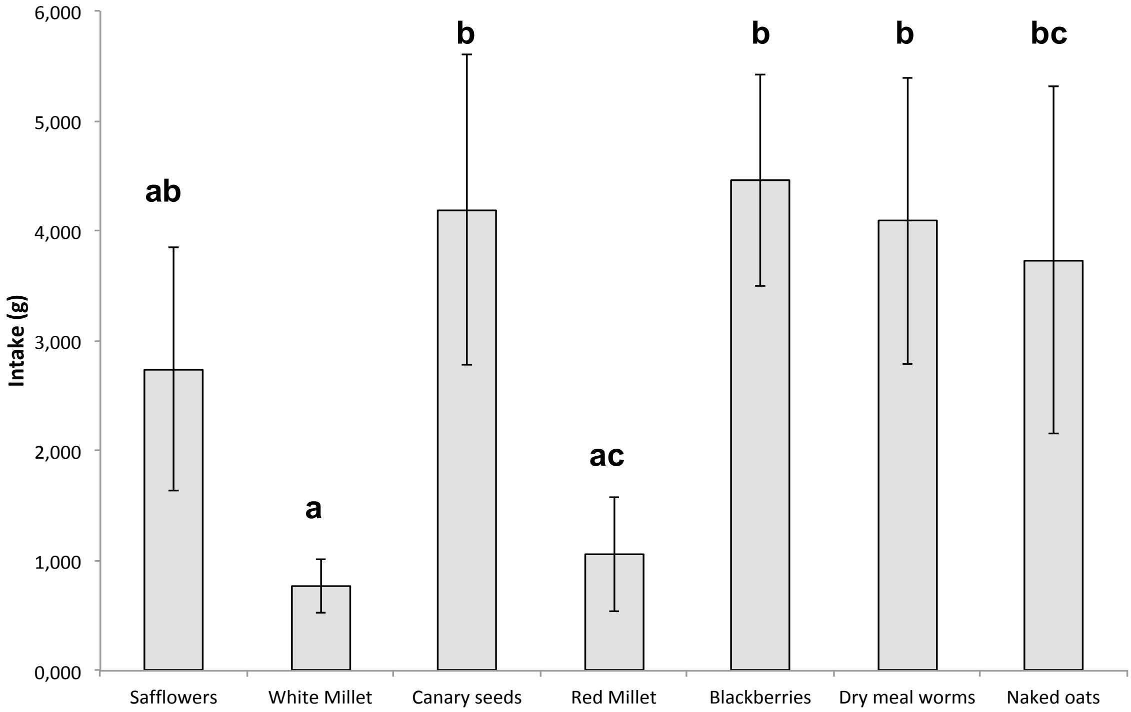

Figure 9: Mean (±S.E.) of food intake (g) for the different food type treatments (safflowers, white millet, canary seeds, red millet, blackberries, dried meal worms and naked oats). See Table 1 for ANOVA parameters. Different letters above bars indicate significant differences according to LSD post-hoc test ($P < 0.05$).

Regarding with food preferences depending on sex (male, female), we can see that males had a clear preference for the dried meal worms (24 %), followed by naked oats (22%), Blackberries (19%) and Canary seeds (19%), showing no cravings for the safflowers (7%). The female in contrast, showed a strong preference for the safflowers (33%), followed by the Blackberries (30%) and the Canary seeds (24%), she did not show any interest in the naked oats (1%) or in the dried meal worms (3%) though. Nevertheless, both males and female barely consumed white and red millet. So, whilst both males and female consumed blackberries and canary seeds, the main difference is that males ate fair amounts of dried meal worms and naked oats, whereas the female seemed to prefer the safflowers (see Figs. 10 and 11).

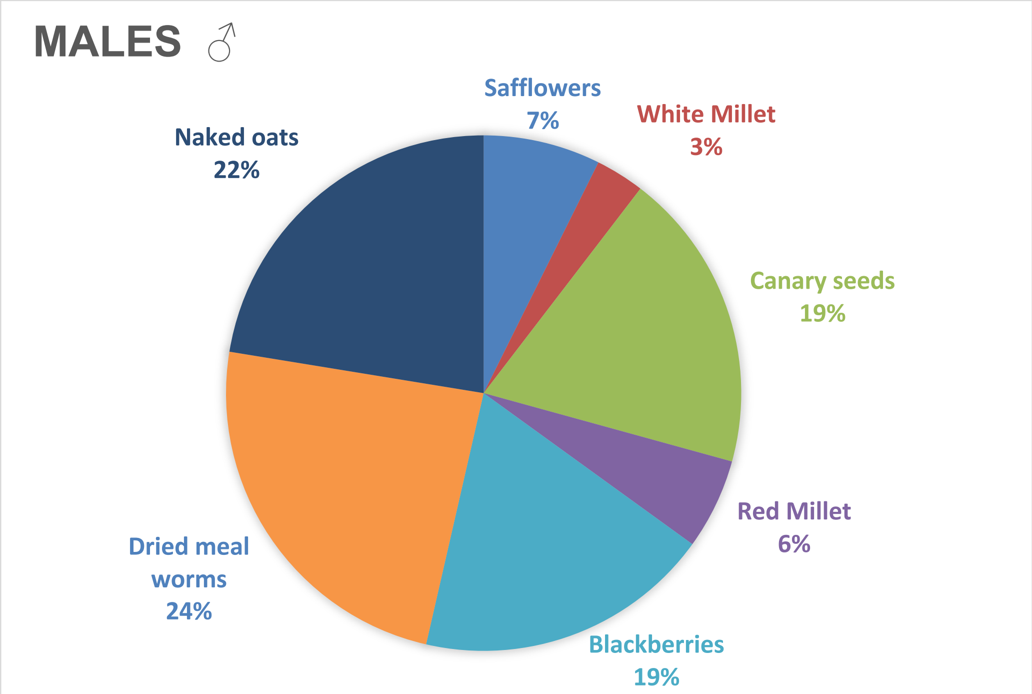

Figure 10. Male percentage of food intake for the different food type treatments (safflowers, white millet, canary seeds, red millet, blackberries, dried meal worms and naked oats).

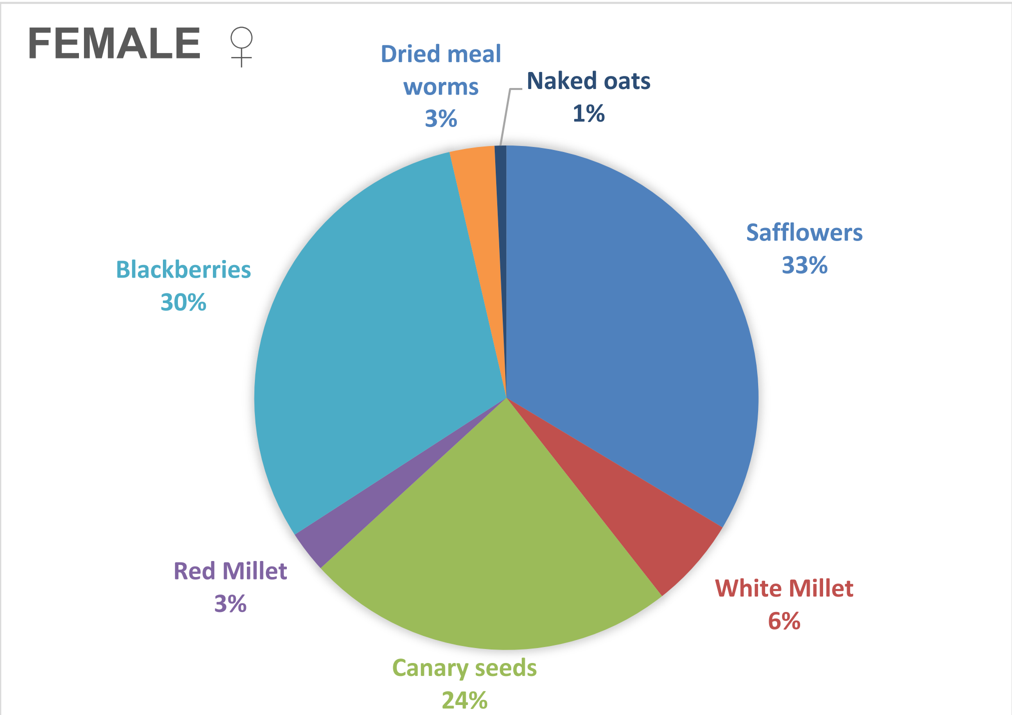

Figure 11. Female percentage of food intake for the different food type treatments (safflowers, white millet, canary seeds, red millet, blackberries, dried meal worms and naked oats).

Food intake differed significantly with feeding time (P-value< 0.001) (see Table 1). The captive Harvest mice that took place in this project showed much higher intakes in the evening and at night (PM mean value = 4.575 g, n = 70) than during the day time (AM mean value = 1.436 g, n = 70) (Fig.12).

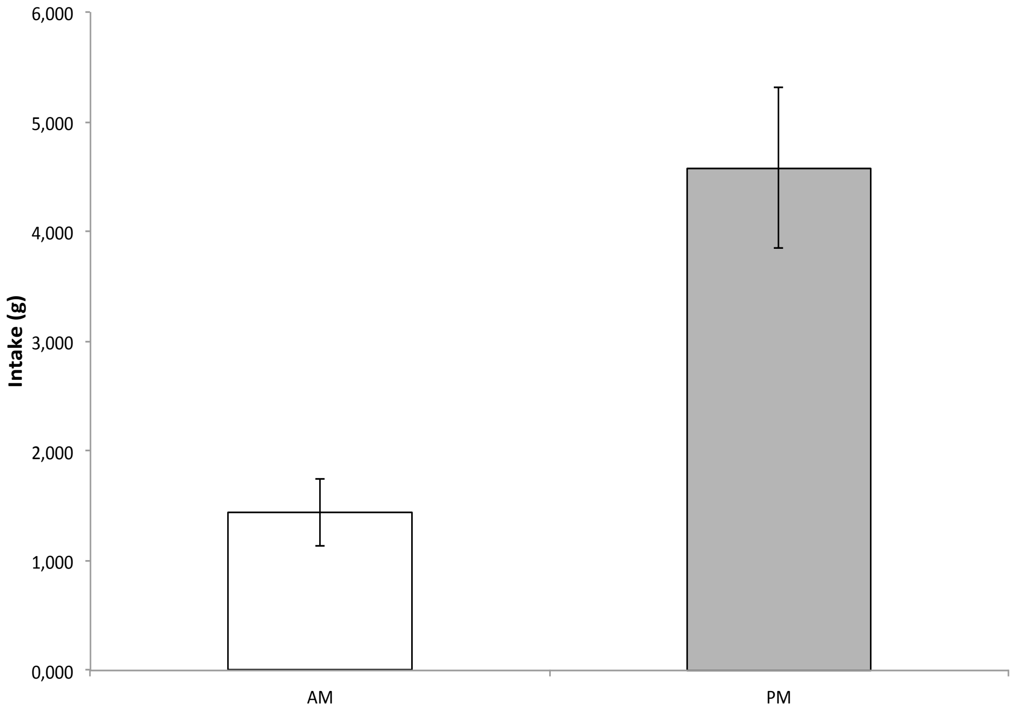

Figure 12. Mean (±S.E.) of food intake (g) for the different feeding time (empty bars for AM, filled bars for PM). AM period consists in times from 8 a.m. to 4 p.m. and PM consist in times from 4:30 p.m. to 8 a.m. See Table 1 for ANOVA parameters.

In spite of the time, the mice still had the same food preferences, since the analysis of the food time preference in regards with the feeding times showed no significant differences (food type x feeding time effect, Table 1). Thus, they still consumed roughly the same proportions of the different feeds both during the AM time and during the PM time (Fig.13).

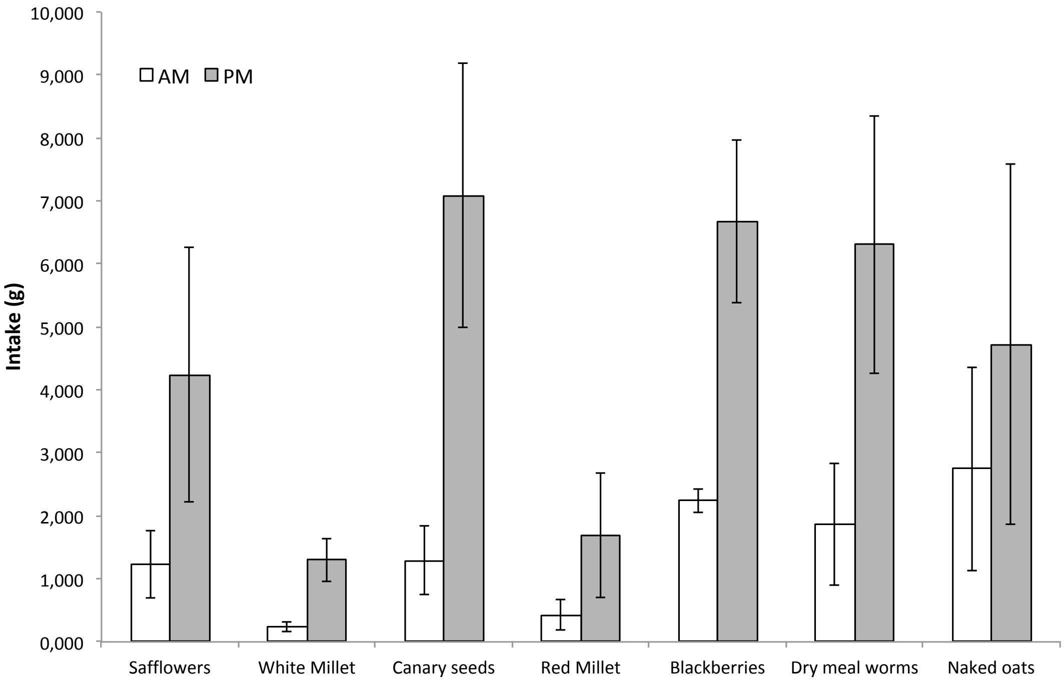

Figure 13: Mean (±S.E.) of food intake (g) for the different food type (safflowers, white millet, canary seeds, red millet, blackberries, dried meal worms and naked oats) and feeding time (empty bars for AM, filled bars for PM) treatments. See Table 1 for ANOVA parameters.

5. Discussion

5.1. Dietary preferences

The preference that mice showed for blackberries can perhaps be explained by their high sugar content in contrast to that of the other feeds and also by their high content in water, especially needed with the high temperatures of the summer season. However, this result may have been influenced by the water evaporation that blackberries may have experienced, which was around 0.1 g per each 2 grams per day, conducting to an erroneous interpretation. The strong preference for canary seeds, dried meal worms and naked oats could be probably explained by protein content, as they constitute the highest source of protein out all the available food options (around 21, 55 and 17 g per 100 g respectively). In addition, canary seeds provide more carbohydrates, while dried meal worms and naked oats provide a greater fat intake. In regard to the safflowers, they also constitute a decent protein supply (around 16 g per 100g) as well as the highest fat supply out of all the tested feeds (around 38 g per 100 g). In view of the results, the millets were the least consumed food items probably due to their low protein content (around 11 g per 100 g) (See Table 2 for nutritional values).

Mice assed in this study were usually given a combination of “Foreign Finch” (containing white millet, panicum millet, red millet and canary seed), “Budgie Mix” (containing red millet, white millet and canary seed) and “Parakeet Mix” (containing stripped sunflower, small sunflower, white millet, canary seed and naked oats in a small proportion). Consequently, in terms of the usual accessibility that mice had to the different food types, white and red millets were a common compound in the food mixes given to the mice and so were canary seeds; whereas blackberries, dried meal worms and naked oats were not accessible for them that often. However, canary seeds were as much accessible for them as white millet, yet they still showed a strong preference for them. So, to a certain extent, the usual accessibility that mice had to the different feeds as well as their personal preferences might have been involved in the results obtained.

Despite the fact that Dickman (1986) found the Harvest mice's diet to be mainly compounded by seeds (45%), leaves (25%), insects (15%) and fruits (6%), this study has showed that whenever mice had the ease and accessibility to fruits (blackberries), invertebrates (dried meal worms) and oats; these would be preferred to certain seeds, although canary seeds were still widely chosen. Thus, the captive Harvest mice that took part in this study preferred certain feeds perhaps as a result of prioritising the fact of achieving a high protein content in their diet.

It is widely known that, as endotherms and due to their tiny body size (high surface to body ratio), Harvest mice have high energy requirements. But, although The Mammal Society (2018) argued that seeds seemed to be the most important item of their diet because of their high energy content, it seems that this is probably more to do with their availability and accessibility that Harvest mice have to them throughout the whole year, as with regard to high energy content the Harvest mice that took part in this project went for the higher protein supplies rather than for the seeds. So, seeds might be the food items that require less energy expense for Harvest and at the same time they present decent energy values worth gaining. However, if the mice did not have to spend high amounts of energy trying to reach and consume blackberries, hunt insect larvae and get rid of the shells that safflowers and oats have, they would probably go for them instead, since they would probably be more beneficial for them in terms of energy. Nonetheless, animals need to measure what is best for them so as to gain the most energy without having to spend it in the process.

Table 2. Energy and nutritional supplies of the different feeds. Data gathered from the National Nutrient Database of the United States Department of Agriculture, Canary seed Development Commission of Saskatchewa and Tesco's wild and domestic bird food and accessories.

	Feeds						
Nutrients/Energy per 100 g	*Safflower*	*White millet*	*Canary seed*	*Red millet*	*Blackberries*	*Dried meal worms*	*Naked oats*
Protein (g)	16.18	11.02	21.67	11.02	1.39	55.2	17.2
Lipids (Fat) (g)	38.45	4.22	5.59	4.22	0.49	25	8.8
Carbohydrate (g)	34.29	72.85	60.93	72.85	9.61	3.6	62.2
Energy (Kcal)	517	378	0.399	378	43	472.6	0.397

5.2. Sex differences

Even though no statistical analysis was applied to analyse the differences presented between sexes due to a lack of female individuals to take part in the study, it is necessary to have a quick glance at the different food preferences showed by males and females before giving any further reasons or explanations for the results.

Both males and female showed agreement on blackberry and canary seed consumptions. However, one possible explanation for the different food preferences showed by the males and female that took part in this study could be that as the project took place in summer (July and August), which coincides with mating season (generally from May until October). Hence, the female could have been trying to increase her fat content in order to be prepared for pregnancy, and as a result added up some extra weight. She actually gained 1.5 g, going from 9 g in weight before the start of the project to 10.5 g at the end. Consequently, she may have preferred to eat safflowers, which had the highest fat and energy supplies out of all the available food items and a decent amount of proteins, and thereby leave out the feeds with higher protein (naked oats and dried meal worms). Males on the other hand, as explained before, preferred the feeds with higher protein content (dried meal worms and naked oats), prioritising proteins over fats and carbohydrates. Accordingly, one possible explanation for it could be the fact that the nutrient requirements of mice are believed to be built on rapid growth leading to maximising body size at maturity. Thus, based on the assumption that a diet promoting growth would be beneficial for reproduction, lactation and maintenance; proteins seem to be the best way of maximising body size (National Research Council (US), 1995). Hence probably why males ate feeds with higher protein content, gaining on average 0.59 g at the end of the project.

In short, it can probably be concluded that the captive Harvest mice that took part in this study showed certain patterns, one of them being a preference for certain feeds as a result of prioritising having a high protein content in their diet. Although when we looked at differences in between sexes, the female showed a clear preference for the feed with the highest fat and energy contents (safflowers) in contrast to that of the males, which preferred the feeds with the highest protein content (dried meal worms and naked oats). Perhaps, this could be explained due to the fact that the female may have been trying to save energy for pregnancy and breeding, whilst males may have been trying to maximise their body size for reproduction purposes. However, as this study did just make use of one female, further studies would have to increase the number of females in order to reach proper conclusions that could support these results.

5.3. Feeding time patterns

We can conclude that individuals had higher activity in between 4:30 p.m. and 8 a.m., coinciding with evening and dusk time, showing in deed a preference for nocturnal behaviour. In spite of that, it also true that Harvest mice are active at both day and night, since day seemed to eat at all times. However, it must be bear in mind again that the study took place in the summer season (July and August), so it confirms what was previously thought about *M.minutus* preferring nocturnal behaviour during summer (Forder, 2006). However, they are also believed to have a diurnal preference during winter. Thereby, further studies to assess their activity during the winter season would be necessary to corroborate this fact.

5.4. Intake rates

Eventually, each mouse ate 3 g per day on average, which constitutes almost a 37% of the total body weight they had before starting the project. So results confirmed what was already known about *M.minutus* eating about a 30% of their own weight each day (Fig. 5) (Oxfordshire Mammal Group, 2018). This result come as no surprise since like it was previously said, Harvest mice have high energy requirements owing to the fact of being endotherms and having a small body size that increases the surface to volume ratio. Thereby, they may experiment huge heat losses that need to be compensated by eating great amounts of high energy foods, reaching up to a 30%-40% of their own weight in food every day.

6. Conclusions

The individuals of captive *M.minutus* that took part in this study showed:

(1) A Preference for blackberries, canary seeds, dried meal worms, naked oats and safflowers over white and red millet.
(2) Differences depending upon sex: Males preferred feeds with higher protein content (Dried meal worms and naked oats), whilst the female preferred the feed with higher fat and energy content (Safflowers).
(3) Higher feeding activity patterns in the evening and at night, stating a preference for nocturnal behaviour.
(4) A 3 g of food consumption per mouse per day on average, constituting up to a consumption of the 37 % of their body weight in food.

Conclusións

Os individuos de *M.minutus* en cativerio que participaron neste estudo mostraron:

(1) Unha preferencia polas moras, o alpiste, os vermes secos, a avea e o cártamo sobre os millos branco e vermello.
(2) Diferencias segundo o sexo: os machos preferiron alimentos con maior contido en proteínas (vermes secos e avea), mentres que as femias preferiron o alimento con maior aportación de graxa e enerxía (cártamo).
(3) Actividades de alimentación máis altas durante a noite, indicando unha preferencia polo comportamento nocturno.
(4) 3 g de consumo de alimento por rato e por día de media, constituíndo un consumo de ata o 37% do seu peso corporal en comida.

Conclusiones

Los individuos de *M.minutus* en cautiverio que participaron en este estudio mostraron:

(1) Una preferencia por las moras, el alpiste, los gusanos secos, la avena y el cártamo sobre los mijos blanco y rojo.
(2) Diferencias según el sexo: los machos prefirieron alimentos con mayor contenido en proteínas (gusanos secos y avena), mientras que las hembras prefirieron el alimento con mayor aporte de grasa y energía (cártamo).
(3) Actividades de alimentación más altos durante la noche, indicando una preferencia por el comportamiento nocturno.
(4) 3 g de consumo de alimento por ratón y por día en promedio, que constituyendo un consumo de hasta un 37% de su peso corporal en comida.

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