Universidade de Aveiro
2021

Inês Franco de Sousa **Os efeitos da alimentação suplementar e da carga ectoparasitária no rendimento reprodutivo do rato-do-campo (*Apodemus sylvaticus*)**

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Acknowledgements

I want to thank all the people who helped me through this journey and for all the support they gave me during this important learning phase.

To my supervisor, Dr Jan Lindstrom, for the opportunity he gave me, for all the guidance, patience and help during the construction of my thesis.

To my co-supervisor, Dr João Carvalho, for his tireless motivation, dedication and mentoring during this phase.

To Dr Simon Babayan, for allowing me to participate in this incredible project and for all the support he gave me during the project. His help was fundamental.

To my parents and sister for their endless patience and support in all important decisions.

To my friends, Tiago Ferreira, Paloma Linck, Ana Elisa Cabral, Miguel Cabral and Olivia Esteves for all the support during this phase and for never leaving my side when I needed them the most.

A huge thank you to you all.

Resumo   A disponibilidade de alimento é um importante factor limitante na natureza. Os seus efeitos na ecologia e história natural das espécies são variados e incluem, por exemplo, a influência na maturação e sucesso reprodutivo individual, a determinação da densidade populacional e a definição das interações entre populações e comunidades. Quando a disponibilidade de alimentos e nutrientes é limitada, a utilização dos recursos é balanceada entre diferentes processos, como o crescimento, a reprodução e a resposta imunitária. O estudo da alocação diferencial de recursos em meio natural constitui um desafio logístico, sendo que a maioria dos estudos realizados são conduzidos em condições laboratoriais controladas, que podem não refletir exatamente as condições ambientais a que os animais estão sujeitos.

Na presente dissertação, realizo uma análise sobre o impacto da disponibilidade de recursos e da carga parasitária em vários parâmetros de desenvolvimento das populações de rato-de-campo (*Apodemus sylvaticus*) na Escócia, cujos dados foram recolhidos por equipas de campo alocadas a um projeto em curso. Foi dada particular atenção à dinâmica entre a disponibilidade de alimento, a resposta imune e a performance reprodutiva.

Os resultados obtidos sugerem que quando os animais se encontram perante uma disponibilidade alimentar superior e uma carga parasitária inferior, registam um aumento da massa corporal e uma resposta imunitária mais eficiente. Esta situação é menos acentuada nos machos, uma vez que o raio de ação mais alargado os deixa expostos a várias fontes de transmissão.   As fêmeas, por sua vez, despendem uma quantidade de recursos e energia significativa na criação da prole, no entanto, tendem a diminuir o seu raio de ação quando os recursos são abundantes.

O estudo foi realizado em meio natural, o que proporcionou resultados que consideramos mais apropriados do que as alternativas puramente laboratoriais, uma vez que o comportamento dos organismos modelo não

foi alterado. A única alteração efetuada em comparação com um meio natural foi o tratamento antiparasitário.

Desta forma, foi possível verificar que as carraças poderão estar a adaptar-se ao ivermectin, conseguindo reproduzir-se mais na presença deste, e que este tratamento tem efeitos negativos na massa corporal dos ratos. O suplemento dado teve efeitos positivos em relação ao peso dos animais, mas quando este era dado em conjunto com o tratamento antiparasitário, houve uma diminuição no número de ratos sexualmente ativos, mostrando uma possível interação negativa entre o suplemento e este tratamento.

Abstract   Food availability is an important limiting factor in nature. The effects of food availability on the ecology and natural history of species include, for example, the influence on individual maturation and reproductive success, the determination of population density and the definition of interactions between populations and communities. When food resources and nutrient availability are limited, the use of resources is balanced between different processes such as growth, reproduction and immune response. The study of differential resource allocation in the wild is a logistical challenge and most of the studies that have been conducted, are carried out under controlled laboratory conditions, which may not exactly reproduce the environmental conditions that wild animals face.

In the present dissertation, I conduct an analysis on the impact of resource availability and parasite load on various developmental parameters of wild wood mouse (*Apodemus sylvaticus*) populations in Scotland, whose data were collected by teams from an ongoing project. Particular attention was paid to the dynamics between food availability, immune response and reproductive performance.

The results obtained suggest that, when the animals are provided with higher food availability concurrently with a lower parasite load, this leads to an increase in body mass and a better immune response. This is less pronounced in males, as their extended range expose the animals to other transmission sources. As females spend extra energy feeding their offspring, they tend to be less mobile when the resources are abundant.

The study was conducted in a natural environment, which provided results that we consider more appropriate than purely laboratory alternatives, as the behaviour of the model organisms was not altered. The only change made compared to a natural environment was the anti-parasitic treatment.

This way, it was possible to verify that the ticks may be adapting to ivermectin, being able to reproduce more in the presence of it, and that this treatment has negative effects on the body mass of the mice. The supplement given had positive effects on the weight of the animals, but when it was given together with the antiparasitic treatment, there was a decrease in the number of sexually active mice, showing a possible negative interaction between the supplement and this treatment.

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1. Introduction

Food availability is an important limiting factor in for wild-living organisms. Its effects on the ecology of species are numerous: from timing of maturation and reproduction at the individual level, to altered population densities, community interactions, and ecosystem functioning (Strandin et al., 2018; Knutie, 2019; Becker et al., 2020). Fundamental among these are the effects of food supply on host-parasite dynamics. The maintenance of immune defence and responses to immune challenges is costly in terms of energy (Pedersen and Babayan, 2011; Strandin et al., 2018; Ferreira et al., 2021). When nutrient availability is limited, it can affect immunity by reducing resources that might normally be spent on alternative processes, such as growth, reproduction, or ornamentation (Rynkiewicz et al., 2019). Most of the studies investigating the health impacts of parasite interactions have been carried out in highly controlled laboratory settings, which often results in limited outcomes, so there is a need for studies in natural environments to evaluate how host-parasite interactions are influenced by external factors and impact host performance (Clerc et al., 2019b). Rodents are an excellent model to study the relationship between parasites and hosts, because they are one of the most successful mammalian groups worldwide, being present in most biotopes on all continents. They have a high reproductive rate and have the ability to adapt to a wide variety of habitats, acting as reservoirs of zoonotic diseases and playing a significant role in maintaining ecosystem functionality as seed dispersing agents and arthropod control (Balčiauskas et al., 2021; Jahan et al., 2021; Koutsoumanis et al., 2021). Rodents are easily sampled, and present short life cycles, this way it's easier to follow all the changes they have throughout life.

Parasites, in turn, are an important part of ecosystems because they can alter the behaviour of their host, its morphology and physiology, as well as influence the role that the host play in the community to which they belong (Khokhlova et al., 2002; Debenedetti et al., 2016). Ectoparasites are vectors for viruses, bacteria, and nematodes, which can cause serious diseases that may eventually affect the reproductive success of the host and consequently its survival and the survival of its offspring (Ponton et al., 2011; Argaez et al., 2020). Parasites, and ectoparasites in particular, can have direct effects, causing tissue damage to the host through allergic reactions. The indirect effects on the hosts are related to changes in reproductive performance, the animal's body condition, its survival and

behaviour, changes in the nutritional needs of the host (Burns et al., 2005; Veitch et al., 2021), as well as changes in the host's immune response (Khokhlova et al., 2002; Reserved, 2018). Host responses to ectoparasites include increased energy allocation to immune defence or changes in their behaviour, which results in energy lost to reduce the number of parasites (Brown et al., 1995; Devevey and Christe, 2009; Bobbie et al., 2017). Consequently, the host can be subject to higher chances of mortality due to poor health, or a decrease in growth, weight or reproductive performance. The natural resistance of hosts, as well as their nutritional state, can influence their immune response (Brouard et al., 2020). To overcome these effects and increase their chances of survival, animals try to accumulate reserves to improve their body condition or decrease their mobility in order to retain the reserves they already have. Animals with better body condition show a decreased risk of starvation in the presence of parasites, a better ability to compete for resources, as well as a better ability to defend themselves from diseases and other parasites (Forbes, 2014). By reducing their mobility, they decrease the risk of predation, and also the chance of getting more parasites due to lessened inter and intraspecific interactions (Díaz and Alonso, 2003; Harrison et al., 2010; Dahmana et al., 2020; de Pelsmaeker et al., 2020).

Generally, males of higher vertebrates such as mammals are infested by more ectoparasites than females (Krasnov et al., 2005; Szentiványi et al., 2017; Medvedev et al., 2020; Hamidi and Bueno-Marí, 2021). Higher relative male parasitism is a complex phenomenon that is related to difference in mobility and home range size, as hypothesised to be a consequence of immunodepressive by testosterone; polygynous males are more active, especially during the breeding season, and have a larger home range than females (Rynkiewicz et al., 2019). This allows them to increase their mating options, although it also increases their exposure to parasitism and the chances of parasite exchange. In contrast, females generally maintain a separate breeding space due to the fact that they are more territorial during this reproductive period, which decreases competition with other females and increases the amount of food per female (Bordes et al., 2012; Hamidi and Bueno-Marí, 2021).

The energy spent on reproduction is generally higher in females than in males, so females that are pregnant or lactating (Jones et al., 2012), may be expected to have stronger responses to the presence of more food than males, who spend the majority of their energy competing with each other (Murray et al., 1998; Harrison et al., 2010). Lactation demands more energy

compared to pregnancy, and parasites end up taking advantage of these individuals who do not have as much energy to fight them. The cost of parasitism can be seen in the parents with reduced mass and survival, as discussed above, or it can be recorded in the offspring, as offspring can be born with reduced body mass and will not grow as much as expected (Moller, 1993; Dlugosz et al., 2014). Immune function is affected by the host's nutrition, which can have a large impact on the outcome of infections. Host nutrition influences both constitutive and susceptible immune function, with consequences on mortality. Secondly, nutrition-based interactions are a major source of microbial benefit to animals. Survival is typically reduced for animals that do not have as much available food, but not for those that are well fed. Maternal antibody transfer can significantly shape litter survival, and is another potential driver of sex differences in immunity (Martínez-Mota et al., 2017; Romeo et al., 2020). Immune memory will reduce the impact of immunopathology for previously observed pathogens (Hasselquist and Nilsson, 2009). Offspring that benefits from maternal immunity will also experience a reduced effect of pathogens on their survival (Patterson et al., 2013; Metcalf and Graham, 2018). During the lactation the additional resource allocation to the embryo will generally exert a cost on the mother, reducing the availability for the mother's own defences. Reproduction also induces a set of physiological and behavioural changes that will indirectly affect susceptibility and exposure to parasites (Albery et al., 2018a; b). This means that simply activating the immune system uses resources that would otherwise keep the animal alive, but when sufficient resources are available, hosts can offset this cost.

Disease tolerance reduces the negative impacts of infection on the host's fitness without reducing parasite burden (Tompkins et al., 1996; Ponton et al., 2013). At the population level, food supplementation or parasite removal has been found to increase the proportion of breeding individuals and allow reproduction during the seasonal reproductive time. However, due to differing competitive abilities, individuals within a population may respond differently to increased food availability or reduced parasitism (Shaner et al., 2018). Most studies investigating the mechanisms of parasite interactions have used highly controlled laboratory environments, which often results in limited outcomes, and it is therefore necessary to conduct trade-off studies in natural environments, to report the magnitude of the impact that these interactions will have on the health of the host (Clerc et al., 2019b). It is becoming clear that multiple factors can be important in driving long-term population

fluctuations, however, few studies have experimentally manipulated several factors at the same time in natural populations to understand how they interact and affect population dynamics (Pedersen and Greives, 2008; Becker et al., 2020). Another problem in these types of studies is the length of observation time, which is often limited to a few weeks. It is important to study these changes over a longer period, from several months to a few years depending on the target species or group, in order to garner statistical support on the biotic and abiotic pressures that drive individual performances and the dynamics of wild populations.

Immunological studies in natural environment are pivotal because immune system responses are context-dependent, varying between laboratory and wild conditions (Jackson, 2015; Rosshart et al., 2019; Graham, 2021). There are several studies that have proven that there is a greater deregulation of the immune system in the laboratory and that this causes a different adaptation of the host to stimuli, either from the environment or from parasites (Rosshart et al., 2019; Graham, 2021). Wild animals have a more complex range of symbioses and exposures to environmental stressors that cannot be equally replicated in the laboratory. By doing these types of studies in the wild, it may be possible to anticipate the risks of infectious diseases that occur in the wild, and perhaps identify variations in the immune defences of animals that occur over time in different generations (Jackson, 2015). My dissertation proposes to better understand the relationship between the parasites and their hosts in their natural habitat. I hypothesize that better access to food will influence the reproduction of wild wood mouse, as well as the presence of parasites, because heavier individuals face better conditions and do not have to balance the use of resources among different physiological demands. Based on Clerc and colleagues (2019c), I carried out a set of experiments in the wild to assess how supplementary feeding and ectoparasite burden affect the reproductive performance of wild wood mouse. Different experiments were conducted to test the hypothesis, in three different forests in Scotland.

2. Materials and methods

2.1. Study Areas

I used data from three experiments (two 8-week experiments and one 32-week experiment: (i) June-July 2015, (ii) June-July 2016 and (iii) May-December 2019), during the breeding season of the wild wood mouse and the weeks after. In 2015/2016 the field experiment took place in Callendar Wood (55.99° N, 3.76° W), and in 2019/2020 it was carried out in Penicuik Park (55.82° N, 3.25° W) and in Hewan Park, in Poulton Vale (55.87° N, 3.14° W)), all in Scotland (Figure 1).

These forests are mostly mature woodlands, with some walking paths. There is a mixture of trees and shrubs, typical of temperate oceanic climate: ash, oak, rowan, and some bramble. All the three sites are very similar in their diversity and vegetation type. The main natural constraints within the woodland for mice populations, are the resource availability and the fact that they are territorial, so they stay within a small range near where they are caught. There are no roads or houses near the sampling places, so anthropogenic disturbance is minimal.
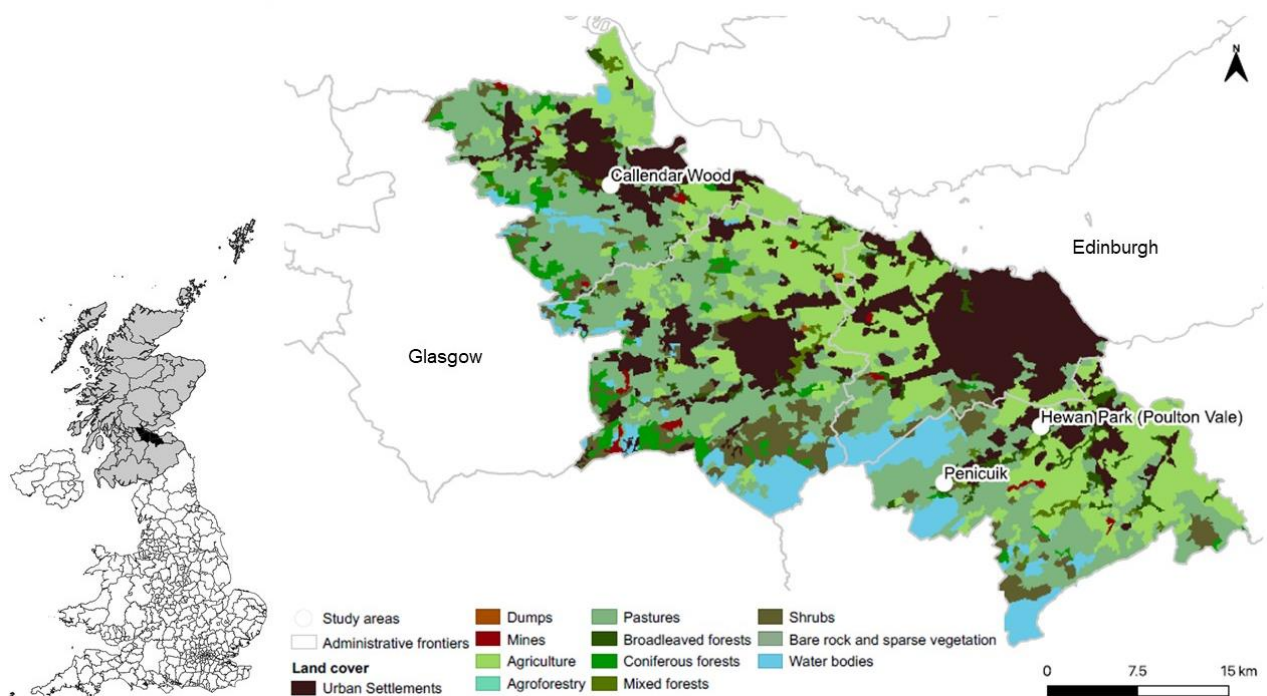

Figure 1 - Location of the three study areas (white circles), depicting the different types of land cover (Copernicus Land Monitoring, 2018).

2.2. Field sampling

Individual wild wood mice were captured using Sherman live traps (5.08x5.6x16.51cm folding trap) (Figure 2) that were placed every 10 meters in a 130x80 m grid (4 grids in 2015/2016 and 10 grids in 2019). In the years 2015/2016, the grids 1, 2 and 3 had 98 traps each, and the grid 4 had 60 traps. In the years 2019/2020, all the grids had 72 traps, except grid 5, where two more traps were set later in the season, making a total of 96 traps in this grid.

Figure 2 - Sherman live traps used in the field work (photograph by Simon Babayan).

All traps were baited. In the supplemented grids, high quality diet (transbreed) was provided by spreading it on the ground (Figure 3). Mice, once they were first captured, were randomly allocated to receive either a single oral dose of a combination of anthelmintic drugs: Ivermectin at 100 mg/kg and pyrantel at 9.4 mg/kg, or an equal volume of water as control, to submit the mice to the same level of stress. At first capture, each wood mouse had a tag injected subcutaneously (AVID FriendChip), which allowed the identification of individuals in the succeeding captures.

Figure 3 - Mouse in the Sherman live trap before treatment and sampling (photograph by Simon Babayan).

Traps were checked every day. At every capture, a record was made of the location of the mouse trap, the sex of the animal, the reproductive status (in the females: vagina perforated, pregnant, or lactating, and in the males: non-reproductive, testicles descended, or scrotal), the body weight, and the body length were recorded. Every individual was examined for the presence of ectoparasites like mites, fleas, and ticks. Collection and analysis of faeces and blood was also performed (Figure 4).

Figure 4 - Material used to collect all the samples needed, like blood and faeces (photograph by Simon Babayan).

2.3. Defining model variables and dataset

The final dataset included 2575 observations of 612 individuals (maximum captures *per* individual =13) in the years of 2019-2020: 1281 in Penicuik Park (where 1034 observations had supplement and 247 acted as control) and 1294 in Hewan Park (where 839 observations had supplement and 455 acted as control). All the mice acted as control for the treatment in both forests. In the years of 2015-2016 the final dataset included 83 observations of 38 individuals (maximum captures *per* individual = 11): where 52 observations have supplement and 31 acted as control, and 42 observations had treatment and 41 acted as control for the treatment (Table 1).

Table 1 - Number of observations *per* study area.

Study areas	Captures (n)	Individuals (n)	Observations with supplement	Observations without supplement	Observations with treatment	Observations without treatment
Penicuik Park	1281	321	1034	247	0	1281
Hewan Park	1294	291	839	455	0	1294
Callendar Wood	93	38	52	31	42	41

2.4. Statistical Analysis

All analyses were performed in R, version 4.1.1 (R Core Team, 2021). We selected the following variables as fixed effects: a) diet type (categorical, two levels: control, supplemented with transbreed), b) reproductive status (categorical, two levels: active [males—scrotal testes, females—lactating or gestating]; inactive [males— descended or abdominal testes, females—perforate or non-perforate vagina]), c) mites (number of individuals observed), d) fleas (number of individuals observed), e) ticks (number of individuals observed), f) body mass, g) treatment (categorical, two levels: control, with treatment (ivermectin) (Table 2).

Table 2 - Fixed effects used in the statistical analysis (all five fixed effects are explanatory variables, but the body mass, ectoparasite burden and the reproductive status are also response variables).

Fixed Effect	Factors (categorical) / Covariates (continuous)	
Diet type	Categorical with two levels	Transbreed
		Control
Reproductive status	Categorical with two levels	Active: females (lactating or gestating), males (scrotal)
		Inactive: females (perforate or non-perforate vagina), males (descended or abdominal testes)
Mites, ticks, fleas	Continuous variable	
Body mass	Continuous variable	
Treatment	Categorical with two levels	With treatment
		Control

For the body mass, we used a linear mixed-effect models (function lmer in R), where the tag and grid were fitted as nested random factors. For the reproductive state, we used a generalised linear mixed-effect test with a binomial error structure (function glmer in R), where the tag and the grid were also fitted as nested random factors. To analyse the counts of ticks, fleas, and mites, generalised linear mixed-effect model was fitted with a negative binomial distribution due to the over-dispersion in the count data of the ectoparasites. Model assumptions were verified by plotting residuals versus fitted values. Model selection was conducted by fitting first the full model with all the explanatory variables and their two-way interactions. A stepwise approach was conducted; the AIC value and the likelihood ratio test were used to select the most parsimonious model (Akaike, 1974).

3. Results

In Callendar Wood, 33 females were observed, 6 of which were pregnant and 9 lactating. From the observations of the pregnant mice, 3 received supplement and treatment, 1 received treatment and no supplement, 2 did not received treatment but did receive supplement, and none of the pregnant acted as control for both treatment and supplement at the same time. For the observations of the lactating mice, 4 received treatment and supplement, 3 received the supplement and did not have treatment, 1 acted as control for both supplement and the treatment, and we did not observe any lactating females with treatment and without supplement. Regarding males, the number of observations was 50, 28 of them in an active reproductive state (scrotal). Seven of them had treatment and supplement, 8 had treatment and acted as control in the supplement, 7 acted as control in the treatment and had supplement and 6 had no treatment and no supplement (Figure 5 and 6). The number of observations in these results are different of the observations in table 1, because here I had to remove all "non available" in this category and retain only the reproductive states identified in the database.

Figure 5 - Number of pregnant and lactating females in Callendar Wood, according with the supplement and the treatment.

Figure 6 - Number of active males (scrotal) in Callendar Wood, according with the supplement and the treatment.

In Penicuik Park, we captured 643 females, where 195 were pregnant and 92 where lactating. 178 of the pregnant acted as control in the treatment and had supplement, and 19 had no treatment and no supplement. Of all the lactating females, 84 acted as control in the treatment and had supplement, and 8 of them had no treatment and no supplement. We captured 197 males in the active reproductive state, where 169 acted as control in the treatment and had supplement, and 28 had no treatment and no supplement (Figure 7 and 8). The number of observations in these results are different of the observations in table 1, because here I had to remove all "non available" in this category and retain only the reproductive states identified in the database.
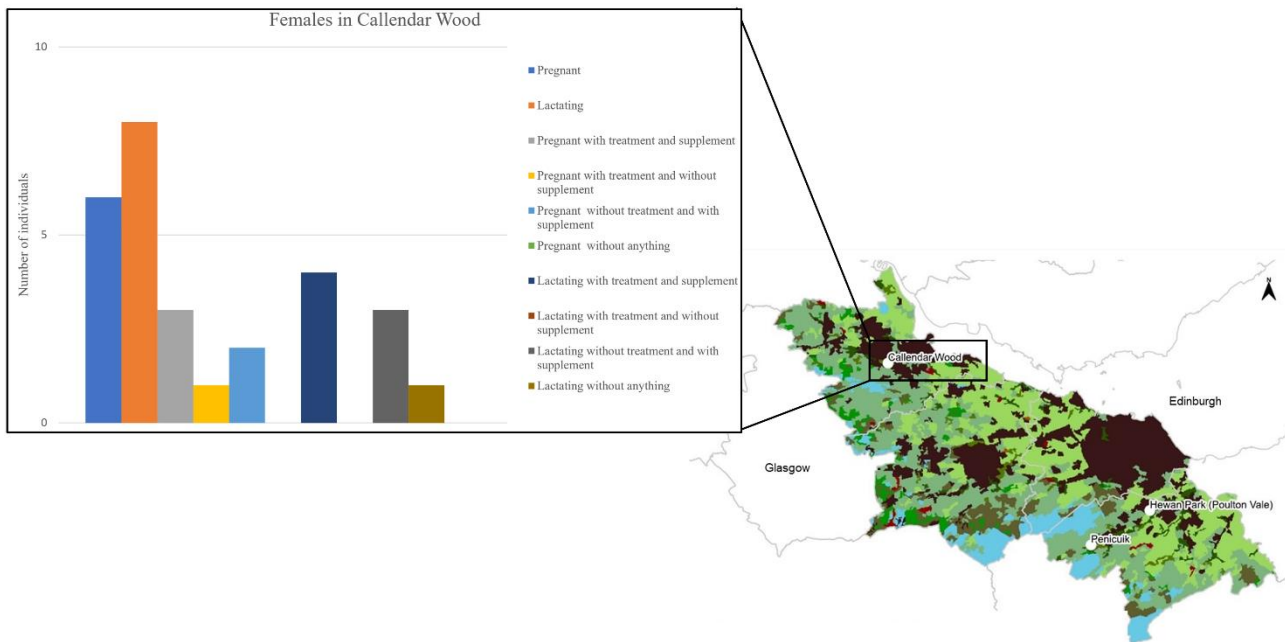
Figure 7 - Number of pregnant and lactating females in Penicuik Park, according with the supplement and the treatment.
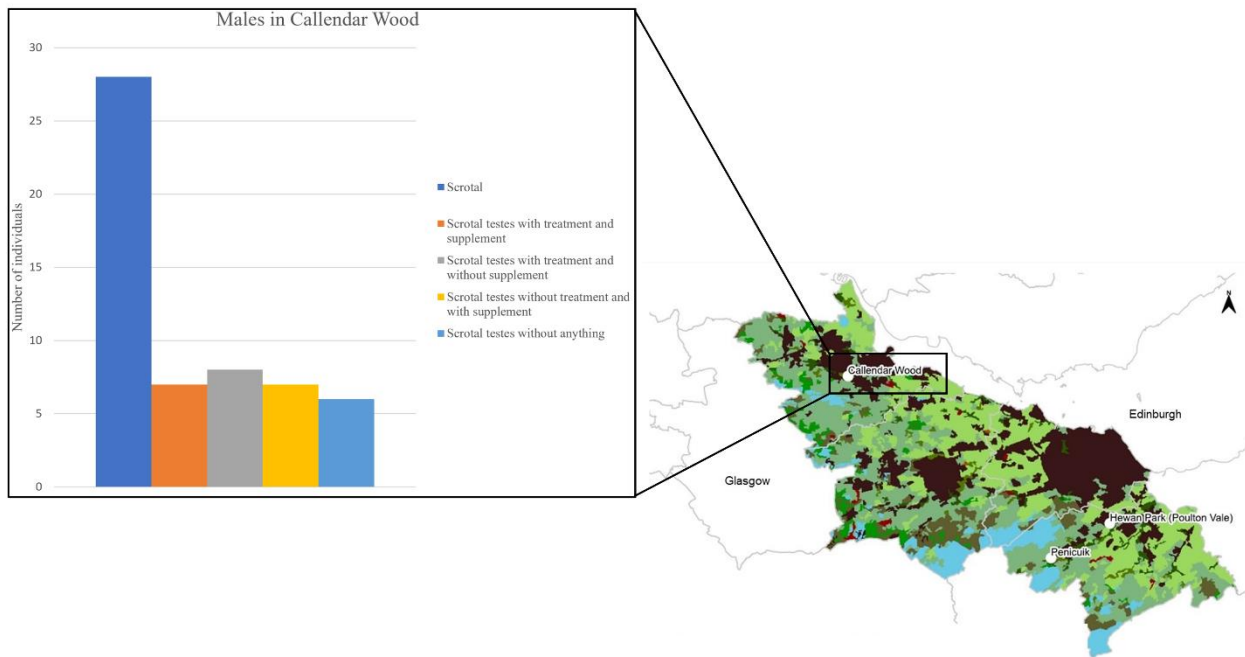

Figure 8 - Number of active males (scrotal) in Penicuik Park, according with the supplement and the treatment.

In Hewan Park, we captured 628 females, 173 of which were pregnant and 76 were lactating. 122 of the pregnant did not have treatment and had supplement, and 51 acted as control in the treatment and in the supplement. Of the lactating females, 55 did not have treatment and had supplement, and 21 acted as control in both situations. We captured 121 males in the active reproductive state (scrotal), where 76 of them did not have any treatment and had supplement, and 45 acted as control in the treatment and in the supplement (Figure 9 and 10). The number of observations in these results are different of the observations in table 1, because here I had to remove all "non available" in this category and leave only the reproductive states identified in the database.
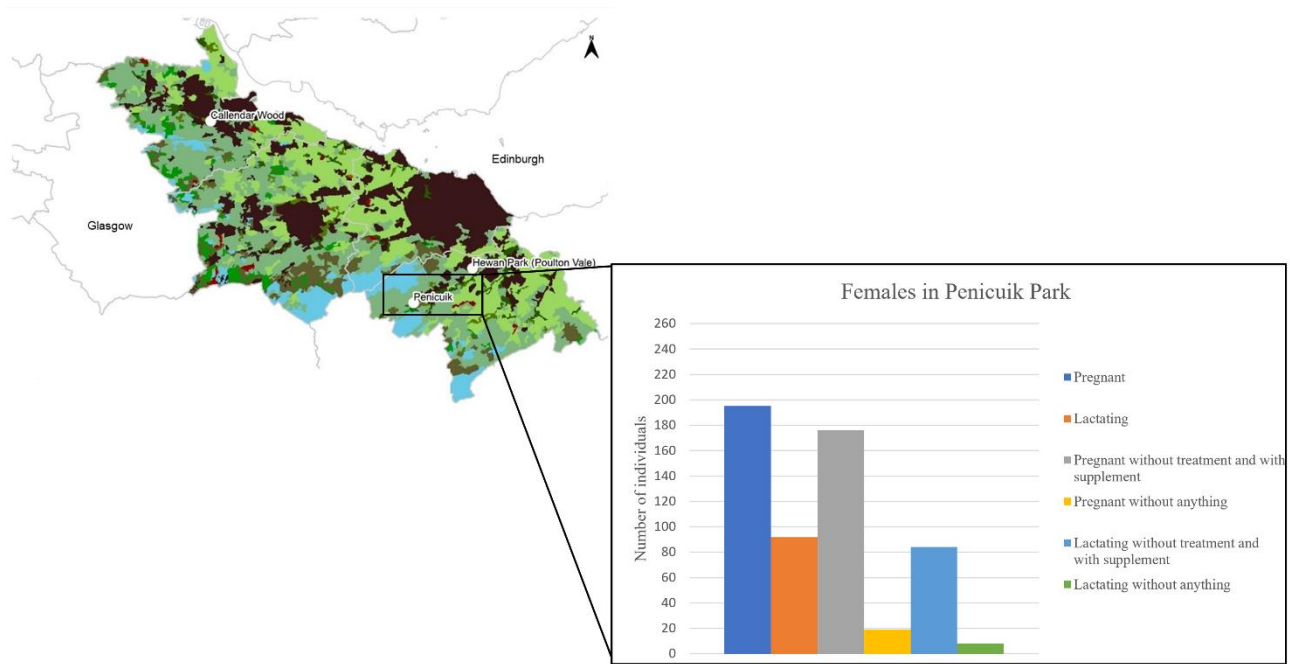

Figure 9 - Number of pregnant and lactating females in Hewan Park, according with the supplement and the treatment.
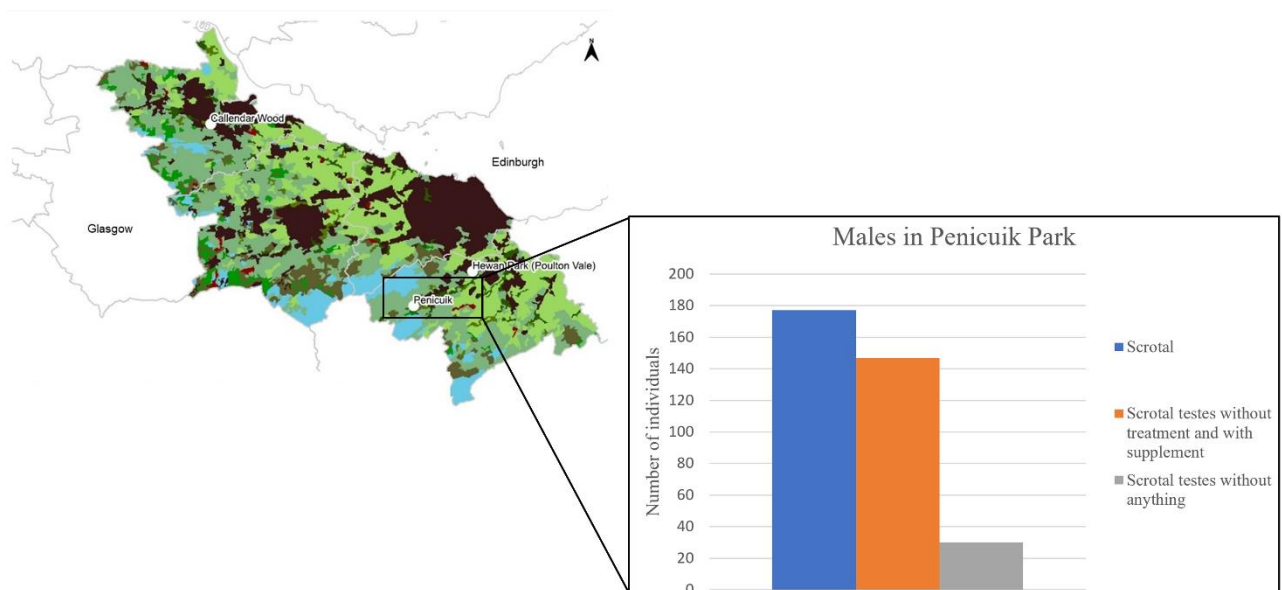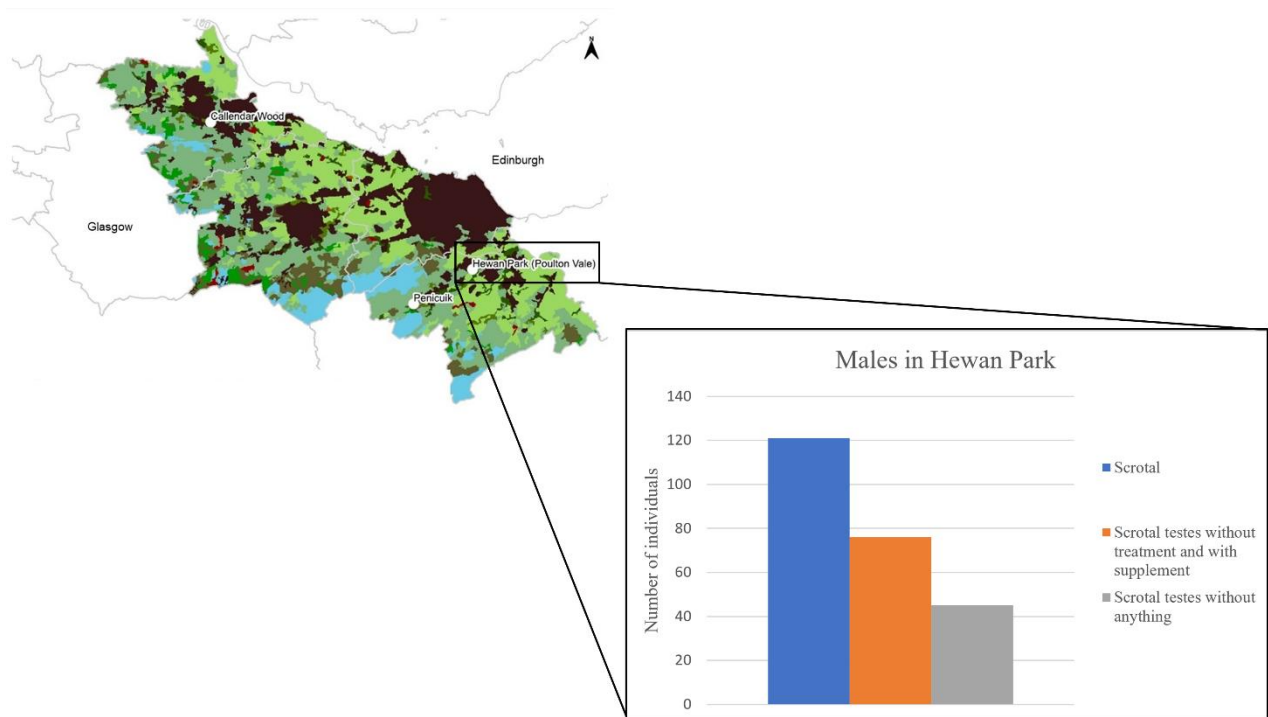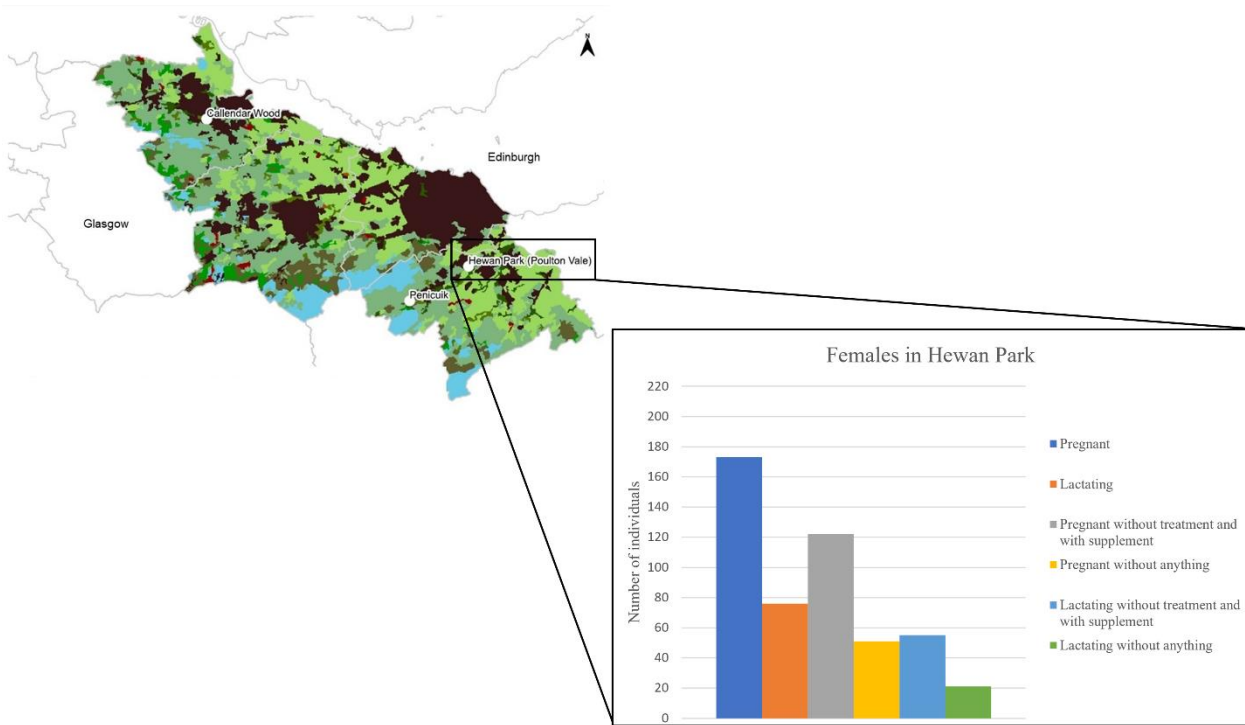
Figure 10 - Number of active males (scrotal) in Hewan Park, according with the food supplement and the parasite treatment.

For the analysis of body mass, the variable of the food supplementation and the active reproductive status (females - pregnant or lactating, and males – scrotal) had a significant positive influence ($\beta_{foodsuppl.} = 1.143$, $p\text{-}value_{foodsuppl.} = {<}0.001^{***}$; $\beta_{reprod.status} = 3.007$, $p\text{-}value_{reprod.status} = {<}0.001^{***}$) and ticks had a negative influence ($\beta_{ticks} = -0.070$, $p\text{-}value_{ticks} = 0.05^*$). For the analysis of active reproductive status, the body mass variable had a positive and significant influence ($\beta_{bodymass} = 0.412$, $p\text{-}value_{bodymass} = {<}0.001^{***}$) being that more pregnant females were observed when the body mass and body condition was higher. The number of ticks and the interaction of treatment and diet (individuals who had treatment and no supplement at the same time) had a positive but not significant influence in the reproductive status ($\beta_{ticks.} = 0.027$, $p\text{-}value_{ticks} = 0.121$; $\beta_{diet\ type\ Control:treatment\ T} = 1.836$, $p\text{-}value_{diet\ type\ Control:treatment\ T} = 0.050$). Diet and fleas had a negative but non-significant influence ($\beta_{foodsuppl.} = 0.234$, $p\text{-}value_{foodsuppl.} = 0.289$; $\beta_{fleas} = 0.144$, $p\text{-}value_{fleas} = 0.443$). For tick count (glm.nb) the three variables tested: treatment, body weight and male sex, had a positive and significant influence ($\beta_{treatmentT} = 1.179$, $p\text{-}value_{treatmentT.} = {<}0.001^{***}$; $\beta_{bodymass} = 0.029$, $p\text{-}value_{bodymass} = {<}0.001^{***}$; $\beta_{sexM} = 0.346$, $p\text{-}value_{sexM.} = {<}0.001^{***}$;). For the

analysis of the number of mites (glm.nb) both treatment and body mass had a negative influence ($\beta_{treatment}$ = -0.515, $p\text{-}value_{treatment}$= 0.094; $\beta_{bodymass}$ = -0.027, $p\text{-}value_{bodymass}$ = <0.001***), with only body mass having a statistically significant value (Table 3). The residuals QQ-plots showed that the residuals of these five variables are all close to the theoretical normal distribution line distribution of the residuals, so all of them followed a normal distribution.

Table 3 - Models used for each response variables, depicting the model estimates and the *p-value* to see which variables are the more significant for each formula.

Formula	Model	Variables	Estimate	P-value
body_mass ~ diet_type + repro + mites	general linear mixed-effect test	**Diet type**	**1.143**	**<0.001 *****
		Reproductive state	**3.007**	**<0.001 *****
		Mites	-0.07	<0.05 *
repro ~ diet_type + ticks_total + fleas + body_mass + diet_type:treatment	generalised linear mixed-effect test with a binomial error structure	Diet type Transbreed	-0.234	0.289
		Ticks total	0.027	0.121
		Fleas	-0.144	0.443
		Body mass	**0.412**	**<0.001 *****
		Diet type Control: treatment T	1.836	0.05.
		Diet type Transbreed: treatment T	0.69	0.391
ticks_total ~ treatment + body_mass + sex	generalised linear mixed-effect model was fitted with a negative binomial error	**Treatment T**	**1.179**	**<0.001 *****
		Body mass	**0.029**	**<0.001 *****
		Sex M	**0.346**	**<0.001 *****
fleas ~ treatment + body_mass	generalised linear mixed-effect model was fitted with a negative binomial error	Treatment T	-1.204	0.267
		Body mass	0.022	0.08.
mites ~ treatment + body_mass	generalised linear mixed-effect model was fitted with a negative binomial error	Treatment	-0.515	0.094.
		Body mass	**-0.027**	**<0.001 *****

These variables were tested between the three forests to verify whether there are significant differences in their values. In the body mass, a boxplot allows a quick visual demonstration of the results (Figure 11). Then, a Shapiro test was performed to test normality and see if it was rejected or not, along with the residuals QQ-plots that showed that the residuals are not close to the theoretical normal distribution line, so normality was rejected, and a Bartlett test to verify the homogeneity of variances, so the homogeneity was also rejected. A Kruskall Wallis test was performed to verify if there is at least one forest with a mean weight significantly different from the others. As the p-value < 0.001, the presence of a forest with significantly different values was confirmed and multiple comparisons were performed using the Bonferroni correction. There are significant differences between Hewan and Penicuik, and between Callendar Wood and Penicuik (Table 4).
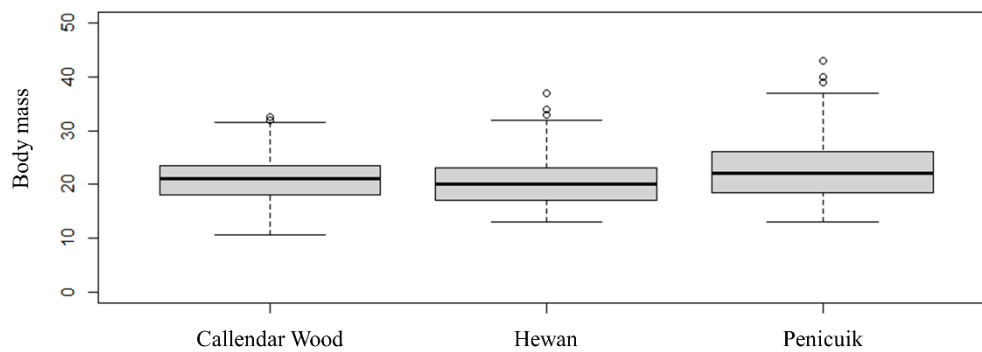

Figure 11 - Box plot of the body mass of the mice in the three forests used in this study.

Table 4 - Mean of the body mass of the mice by each forest (n=number of individuals).

	Callendar Wood (n=235)	Hewan (n= 1294)	Penicuik (n=1280)
Mean of the body mass (gr)	20.68 ± 0.28	20.17 ± 0.11	22.36 ± 0.18

For the ticks, we also made a box plot initially (Figure 12), followed by a Shapiro test to verify the normality and see if it was rejected or not, along with the residuals QQ-plots, that showed that the residuals are not close to the theoretical normal distribution line, so normality was rejected. And a Bartlett test to verify the homogeneity of variances where the homogeneity was also rejected. A Kruskall Wallis test was performed to verify if there is at least one forest with a number of ticks significantly different from the others. As the *p-value* < 0.001, the presence of a forest with significantly different values was confirmed and multiple comparisons were performed using the Bonferroni correction (Table 5).
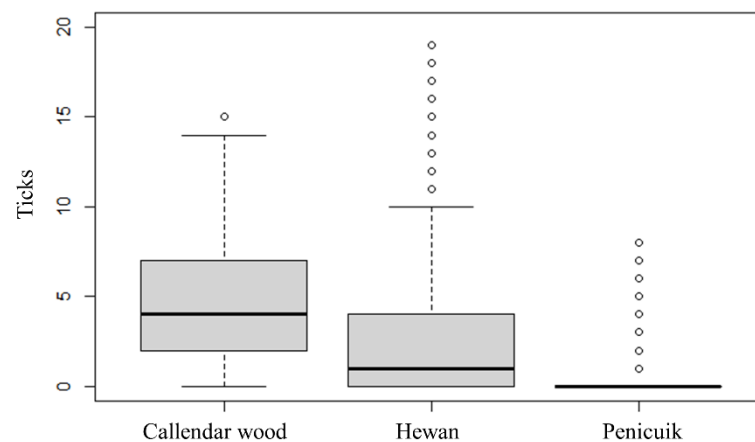
Figure 12 - Box plot of the number of ticks in each forest.

Table 5 - Mean of the number of ticks in each forest.

	Callendar Wood	**Hewan**	**Penicuik**
Mean of the number of ticks	4.92	2.57	0.43

For the mites, a box plot was made (Figure 13), followed by a shapiro test to verify the normality, along with the residuals QQ-plots that showed that the residuals are not close to the theoretical normal distribution line, so normality was rejected, and a Bartlett test to verify the homogeneity of variances, so the homogeneity was also rejected. A kruskall wallis was performed to verify if there is at least one forest with a number of mites significantly different from the others. As the p-value < 0.001, we verified that there was a significant difference between the forest in relation of the number of mites (Table 6).
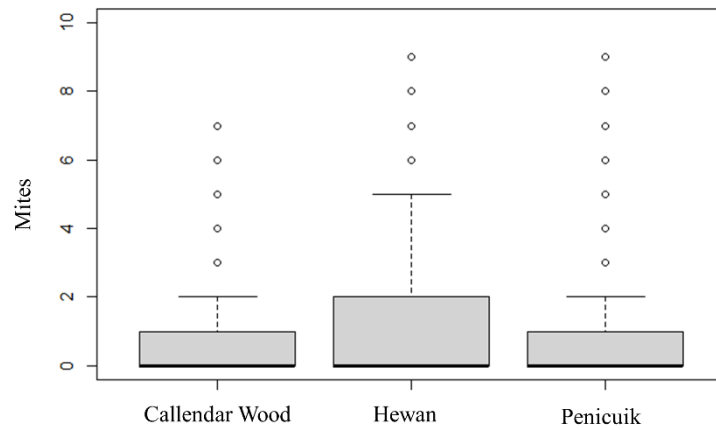
Figure 13 - Box plot of the number of mites per forest.

Table 6 - Mean of the number of mites per forest.

	Callendar Wood	**Hewan**	**Penicuik**
Mean of the number of mites	0.94	1.36	0.97

For the reproductive state, we started by checking the number of sexually active mice (represented by 1) and the number of non-active mice (represented by 0) (Table 7), and then we did a Shapiro test to check the normality of this variable, along with the residuals QQ-plots that showed that the residuals are not close to the theoretical normal distribution line, so normality was rejected. We did a pairwise comparisons for proportions test to make multiple comparisons of the proportions in each forest (Table 8).

Table 7 - Number of active and non-active mice per forest.

Reproductive state	**Callendar Wood (n=235)**	**Hewan (n=1262)**	**Penicuik (n=1265)**
0	92	892	801
1	143	370	464

Table 8 - Comparisons for each combination of forests related to the reproductive state.

Group 1	Group 2	P-value adjusted
Callendar Wood	Hewan	5.25e-20 ****
Callendar Wood	Penicuik	1.37e-11 ****
Hewan	Penicuik	9.92e- 5 ****

4. Discussion

I investigated the impact of a supplemented feeding and ectoparasite burden on the reproduction of the wild wood mouse, in a wild context with as little anthropogenic interaction as possible, in three different locations in Scotland. The results demonstrate a significant benefit of transbreed diet in the majority of the mice, both reproductively active and inactive, which is in line with previous findings (Díaz and Alonso, 2003 and Sweeny et al., 2021,Appendix A). I demonstrated that an enriched diet increases the weight of the mice, which consequently increases the animals' immunity, and that ends up reducing the infection by mites and probably it's transmission to other individuals (Appendix B). Surprisingly, the results also demonstrated that higher body mass, together with the treatment for ectoparasites, had a positive effect on the number of ticks (they reproduce faster in mice in higher body condition and with treatment) (Appendix C). This is a result that needs to be carefully analysed to assess if this treatment is not the best option to fight ticks, if the ticks are gaining resistance or mutating against this treatment or if it is a statistical problem resulting from the large difference between the number of individuals treated with ivermectin and the control. The unbalanced ratio between both groups may influence the final results.

The treatment did not show a significant impact in the burden of fleas and mites, although a reduction on the number of the ectoparasites was expected, even if minimal. Clerc et al., 2019a, say that ten days after the treatment is given, it starts to lose effect and the parasite numbers start increasing again, which can explain why the number of mites and fleas did not suffer a significant decrease in number. The best option to see a decrease in the number of this parasites would be to give the treatment with 10 days of interval. I demonstrated that males are characterized by a higher parasite burden, which can be explained by their extensive foraging, as well as larger home range, which increases their exposure to parasitism (Sánchez et al., 2018 and Hamidi and Bueno-Marí, 2021). The results confirm that individuals with higher body mass are the ones with better conditions to produce more offspring, corroborating previous findings (Sweeny et al., 2021 and Appendix D).

Here, ivermectin administration could be linked to the loss of host weight. This fact can affect the survival of the individuals and their capacity of producing offspring (Appendix E). When the ivermectin is given along with the supplement (transbreed), the number of reproductive active individuals had a decrease when compared to the mice that only had the

ivermectin without the transbreed diet (Appendix F), and it was expected an increase in the number of individuals when they had supplement and treatment for ectoparasites, and not the opposite. This suggests that there is a negative interaction between the treatment and the supplement. This is not in line with Lo and Shaner, 2015 and others studies, where they concluded that the treatment even helped the individuals to gain weight, especially in the reproductive females, which is why it's important to repeat this experiment with more treated mice to check if is not a statistical problem or if it is in fact an important problem with the ivermectin.

In the last tests performed, I found that Penicuik has a significant difference in the weight of mice compared to the other two, which may lead to think that the vegetation of the forest may be slightly different from the others, which ends up nourishing more mice along with the transbreed given. There were significant differences in the three forests in relation to ticks, which can be explained by the large difference in the number of individuals in each forest that may end up influencing the final result, as well as the very small number of treatments in the total of observations. Callendar Wood was the one that obtained the highest number of ticks present on the mice, which is curious because the mice of this forest were the only to received treatment for ectoparasites and were expected to have less ectoparasites. These results may have been influenced by it, being the forest with the smaller number of observations collected compared to the other two (almost 1000 observations less), eventually influencing the average *per* mouse, but may also be related to the treatment not having positive effects on the mice making them more susceptible to ticks. The ideal situation would be to repeat this study, with a more similar number of observations between the forests and with treatment for ectoparasites in all locations, to verify if these results are due to statistics or if in fact there is some mutation in the ticks in relation to the treatment given.

Regarding the mites, Hewan was the forest that had more significant results compared to the other forests. This may be related to the fact that the mice in this forest were the ones that recorded lower weights, which eventually caused the mice to have weaker immunity, and could therefore not fight the mites so well. We should control for the confounding effects of habitat by performing a detailed description of habitat composition and resources availability. Regarding reproductive status, there is a significant difference between the number of active to non-active mice in the three forests, with Callendar Wood having more active mice, even though it had the presence of more ticks, and the other two having more

non-active mice. The fact that there are few observations in this wood, may influence the final statistical values. Being a smaller group and having more active mice with more ticks, there may be an adaptation on the part of the mice, both in relation to the environment surrounding them and thus ending up being able to maintain a balance between immunity and reproduction. This also can be explained by the fact that by existing more individuals sexually active, there are more interactions between them, and they are more expose to a larger number of ticks.

5. Conclusions

In this study we demonstrated that an enriched diet increases the weight of the mice, which consequently increases the animals' immunity. Our results also show that higher body mass along with ivermectin had a positive effect on the number of ticks. This result needs more attention, to study alternatives to the used treatment for ticks, or if the ticks are gaining resistance to this treatment.

Also, the ivermectin could be linked to the reducing of the host weight, which could affect survival and capacity of producing offspring, because when the ivermectin is given with the supplement (transbreed) the number of reproductive active mice had a decrease when comparing to the mice that only had the ivermectin, without the transbreed. This situation needs to be studied, to see if it was a statistical error due to there being few mice treated for ectoparasites that influenced the final result, or if in fact there was some negative effect of the treatment or its interaction with the food given to the mice.

A final suggestion is to address these questions with a number of mice treated with ivermectin and/or supplement compared to a similar number of control mice. By using a balanced design, we can verify the efficiency of this treatment against parasites, mainly against ticks, and to what extent this treatment influences individual performance. This way, we can see if this supplement and treatment can be used in other species that need to increase their reproductive performance, or if we need to change the treatment used in this study because of the possible toxic effects our results suggested.

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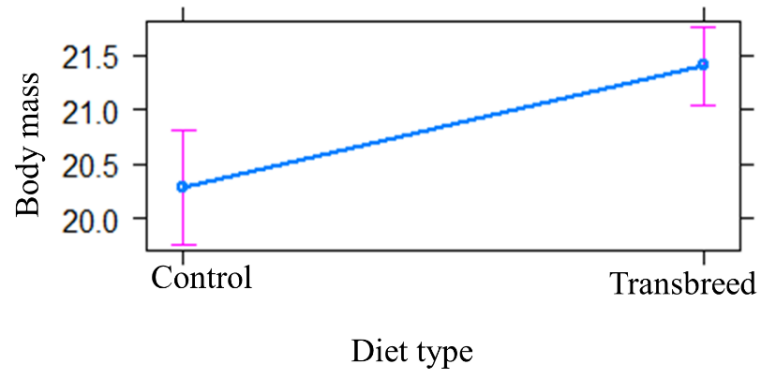
7. Supplementary Data

7.1. Appendix A

Figure 1 - Relationship between the body mass and the transbreed diet given to the mice.
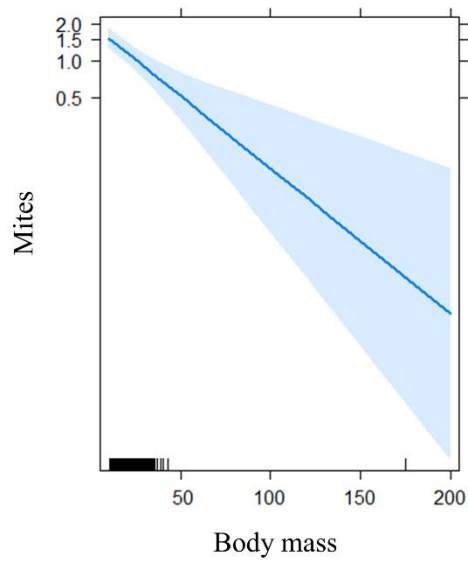
7.2. Appendix B

Figure 2 - Plot relating the number of mites to the weight of the mice, showing that if the weight increases the number of mites decreases.
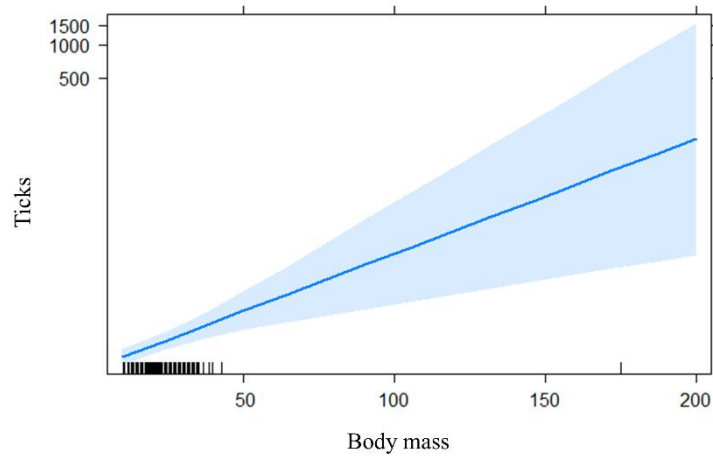
7.3. Appendix C

Figure 3 - Relationship between the number of ticks and the weight of the mice, showing that if the weight increases the number of ticks also increases.
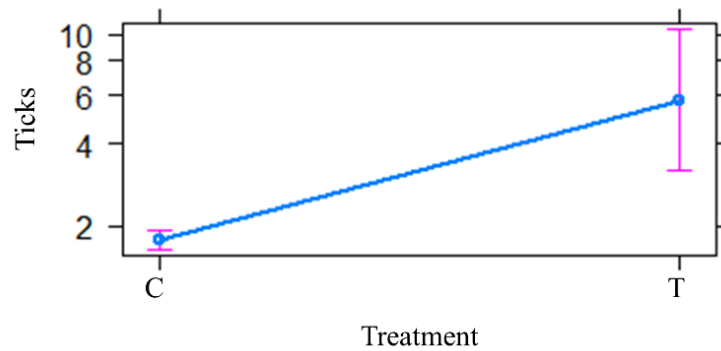

Figure 4 - Plot of the relationship between the number of ticks and the presence/absence of treatment against ectoparasites, when in the presence of treatment we have an increase in the ticks.
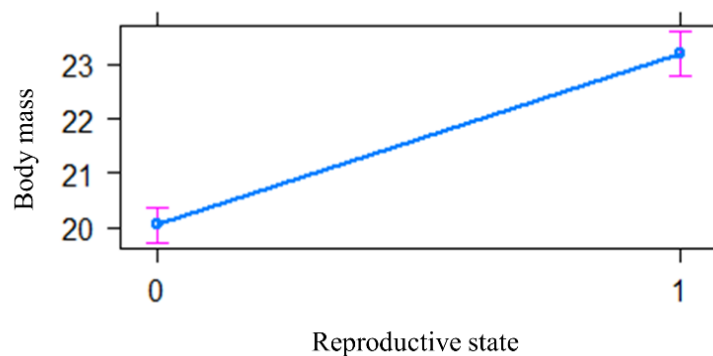
7.4. Appendix D

Figure 5 - Difference between the weights of reproductively active and inactive mice.
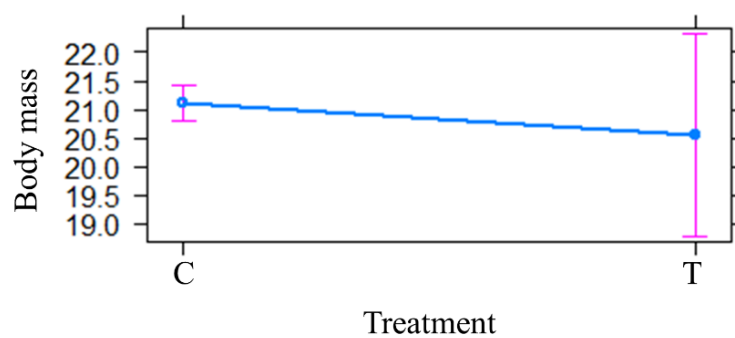
7.5. Appendix E

Figure 6 - Difference in the body mass of the mice with and without treatment for the ectoparasites.
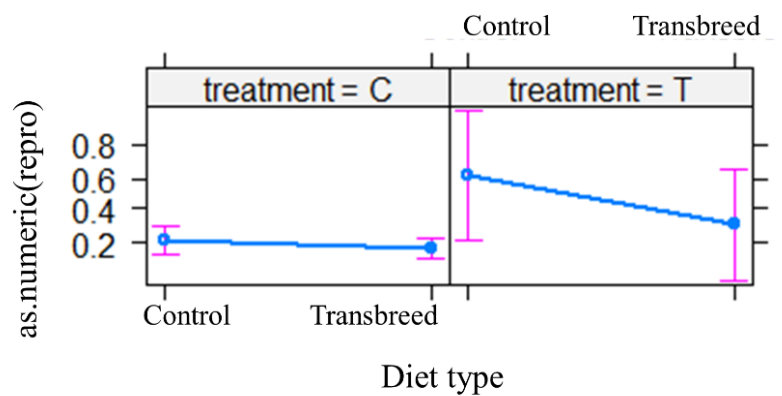
7.6. Appendix F

Figure 7 - Effects of the interaction of the treatment of the ectoparasites with the transbreed diet on the number of reproductively active mice.