Inter-individual differences and parasites: effects of host behavior, space use, and physical traits on ectoparasite loads in the yellow-necked mouse (*Apodemus flavicollis*)

Różnice międzyosobnicze a pasożyty: wpływ zachowania, użytkowania przestrzeni i cech fizycznych żywiciela na liczebność ektopasożytów myszy leśnej (*Apodemus flavicollis)*

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ABSTRACT

Parasites often exhibit a pronounced aggregation among their hosts, but the mechanisms behind these patterns remain elusive. It is believed that inter-individual differences between the hosts – among them sex, body mass, and consistent behavioral differences referred to as 'animal personalities' – play a role in these host-parasite interactions. For instance, males have been observed to have higher parasite loads, possibly due to a testosterone-related immune handicap or a stronger tendency to roam in parasite-contaminated habitat than females. Moreover, animals that show higher levels of exploration in 'open-field tests' (which are thought to reflect their activity in natural habitat) might also be at greater risk of parasitism. To investigate this subject, I conducted live-trapping, repeated behavioral tests, parasite collection, and experimental parasite reduction in a wild population of yellow-necked mice, *Apodemus flavicollis*.

In the first part of my dissertation (Chapter I), I examined the tick and flea burdens of male and female mice, and investigated whether these burdens were influenced more by sex or body mass. I found that male mice had more ticks than females. However, this disparity was due to differences in host body mass rather than sex itself. I observed a significant positive correlation between host body mass and flea loads, but there was no indication of sex-based differences in flea abundance.

In the second part of the dissertation (Chapter II), I investigated the feedbacks between a host's behavior, space use, and ectoparasite loads. The data revealed that the relationship between exploration in the open field and trapping-derived indices of space use was more complex than initially predicted. Specifically, while open field exploration was positively correlated with trap overlap (often used as an index of sociability), the relationship between exploration and trappability (used as an index of boldness) was not linear, but U-shaped, and there was no relationship with the average distance between captures (an index of mobility). The antiparasitic treatment effectively reduced ectoparasite abundance, but this reduction did not affect open field exploration. Among space use indices, only trappability increased in response to the antiparasitic treatment.

In summary, I explored the inter-individual traits of a wild population of yellow-necked mice, both physical – such as body mass and sex, and behavioral – personality and space use, to assess how they affect ectoparasite infestation. In addition, the complex interactions between mouse behavior, space use, and the level of ectoparasite infestation not only suggest the existence of a negative feedback loop, but also challenge the commonly proposed mechanism about how individual differences affect space use, and therefore host-parasite interactions.

Keywords: Ectoparasites, sex, body mass, animal personality, space use, negative feedback

STRESZCZENIE

Pasożyty często gromadzą się u pewnych żywicieli bardziej niż u innych, ale mechanizmy leżące u podstaw tego zjawiska pozostają w dużej mierze niejasne. Wewnątrzgatunkowe różnice pomiędzy żywicielami, w tym płeć, masa ciała oraz różnice behawioralne, określane jako ‘osobowość zwierząt’, są uznawane za czynniki mogące kształtować interakcje między żywicielem a pasożytem. Badacze przypisują wyższe obciążenie pasożytami samcom, w konsekwencji ich osłabionej odporności lub silniejszej w porównaniu z samicami tendencji do eksplorowania środowiska zanieczyszczonego pasożytami. Ponadto wyższy poziom eksploracji, mierzony w arenie eksperymentalnej (przy założeniu, że wyniki testu odzwierciedlają aktywność zwierzęcia w jego naturalnym środowisku), może również wpływać na ryzyko zarażenia pasożytami. Aby zbadać te kwestie, przeprowadziłam odłowy, wielokrotne testy behawioralne i ocenę stanu zapasożycenia dzikiej populacji myszy leśnej (*Apodemus flavicollis*).

Po pierwsze, porównałam poziom zapasożycenia kleszczami i pchłami samców i samic myszy leśnej oraz sprawdziłam, czy różnice były spowodowane płcią żywiciela, czy jego masą ciała (Rozdział I). Odnotowałam większe obciążenie kleszczami u samców niż u samic. Jednak ta różnica była spowodowana różnicami w masie ciała, nie zaś płcią jako taką. Zaobserwowałam pozytywny związek między masą ciała żywiciela a liczbą pcheł, lecz nie było różnic w liczebności pcheł w zależności od płci.

Po drugie, zbadałam wzajemne związki między zachowaniem żywiciela, a ich obciążeniem ektopasożytami (Rozdział II). Związek między wynikami testu otwartego pola a użytkowaniem przestrzeni był bardziej złożony, niż zakładały moje przewidywania. Stwierdziłam pozytywną korelację między wskaźnikiem socjalności a eksploracją w otwartym polu, podczas gdy prawdopodobieństwo złowienia wykazało U-kształtny związek z eksploracją. Kuracja przeciwpasożytnicza skutecznie zmniejszyła liczbę ektopasożytów, ale nie wpłynęła na wyniki testu otwartego pola. Spośród wskaźników użytkowania przestrzeni tylko prawdopodobieństwo złowienia wzrosło po zastosowanej kuracji przeciwpasożytniczej.

W badaniach wykazałam, że różnice zapasożycenia u myszy leśnej wynikają z różnic masy ciała, nie zaś różnic między płciami. Ponadto, złożone interakcje między zachowaniem myszy, użytkowaniem przestrzeni oraz poziomem infestacji ektopasożytami nie tylko sugerują działanie negatywnego sprzężenia zwrotnego, ale także kwestionują często proponowany mechanizm dotyczący tego, jak zachowania żywiciela wpływają na użytkowanie przestrzeni, a przez to na interakcje żywiciel pasożyt.

Słowa kluczowe: ektopasożyty, płeć, masa ciała, osobowość zwierząt, użytkowanie przestrzeni, negatywne sprzężenie zwrotne

INTRODUCTION

The distribution of parasites among hosts is commonly understood to follow non-random, aggregated patterns, but the reasons for this phenomenon are still poorly understood. Several potential explanations center on inter-individual differences within host populations, encompassing factors such as sex, body size, and behavioral traits. (Krasnov et al. 2012, Barber & Dingemanse 2010). Yet, the underlying mechanisms of parasite aggregation remain ambiguous.

One of the commonly observed biases in parasite distribution is attributed to sex differences between individuals. Male bias has been observed in many bird and mammal species. However, the causes of this pattern are still being debated, partly because of the difficulties in identifying the driving factor, if it is correlated with other traits, such as host body mass (Morand et al. 2004).

Other widely discussed factors that are believed to shape parasite loads are the personality and spacing behaviors of the host (Barber 2010, Sih et al. 2018). As most parasites are acquired by the host from the environment or its conspecifics, personality, (defined as consistent behavioral tendencies, stable across time and contexts: Sih et al. 2004) can affect how animals move around in their environment, and over time leads to bias in parasite acquisition. Animal personalities are typically assessed in standardized behavioral tests under the assumption that such tests reflect ecologically relevant behavior in the wild. For example, animals that are more exploratory in widely-used open-field tests may be more likely to become infected with parasites if the test scores reflect hosts' activity in the natural habitat. In addition, possible contact rates between individuals, affected by animal sociability levels, are also known to affect parasite transmission (Habig & Archie, 2015). However, host behavior can also be modified by the presence of parasites, further complicating our understanding of such dynamic systems.

In my dissertation, I explored these problems using a wild population of yellow-necked mice (*Apodemus flavicollis*) and their ectoparasites. Rodents are important hosts for a wide variety of parasites, which in turn can be vectors of diseases of humans, pets, and livestock (Ecke et al. 2022). Therefore, studying the trait-driven dynamics of parasite infestations is of interest not just for ecologists, but also epidemiologists. To explore the reciprocal links between hosts traits and their ectoparasite loads, we live-trapped the mice, conducted repeated behavioral tests, harvested parasites, and conducted an experimental manipulation involving antiparasitic treatment.

The yellow-necked mouse is a rodent species with evident sexual size dimorphism, making it an ideal model to explore questions related to the interplay between body mass and sex. In Chapter I of my dissertation, I built upon existing research of the sex-ectoparasite link in a related species, *Apodemus sylvaticus* (Harrison et al. 2010). I conducted a quasi-replication of this study and compared the effect sizes observed in both species. My results indicate that the male bias in parasite loads in these species is driven primarily by the differences in the body mass, not by sex itself.

In Chapter II, I investigated the effects of personality and spatial behavior on ectoparasite loads in the yellow-necked mouse. This was achieved through a series of repeated personality tests and ectoparasites surveys. However, while behavior may affect parasite loads, it is also recognized that parasites can, in turn, affect host behavior (Moore 2002, Ezenwa et al. 2016). Therefore, to disentangle these possible effects, a mere observational study is not sufficient. Thus, I conducted an experimental parasite reduction to address the theory of a negative feedback loop between host behavior and its ectoparasites. Corroborating this theory, I found indications that infested mice demonstrated reduced trappability, which increased after the antiparasitic treatment. However, many anticipated connections between behavior, space use, and ectoparasite loads were not confirmed, suggesting potential gaps in the prevailing theory.

In summary, I explored individual traits that shape ectoparasite loads in the yellow-necked mouse, an abundant, widely distributed rodent. This species not only plays a pivotal role in many ecosystem functions (Jędrzejewski & Jędrzejewska 1993, Zhang et al. 2003, Stephens & Rowe 2020, Godó et al. 2022), but also holds significant importance in the epidemiology of zoonotic diseases (Ecke et al. 2022). I discovered a reassuring consistency with previously reported patterns where body mass drives sex bias in *Apodemus* ectoparasite loads. However, there was a less comforting discrepancy between my findings and the theoretical mechanism where behavior affects parasite loads through host space use. Additionally, I highlighted a previously unreported negative feedback loop between certain aspects of host behavior (specifically trappability) and parasite burdens.

REFERENCES

Barber, I., & Dingemanse, N. J. (2010). Parasitism and the evolutionary ecology of animal personality. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1560), 4077-4088.

Ecke, F., Han, B. A., Hörnfeldt, B., Khalil, H., Magnusson, M., Singh, N. J., & Ostfeld, R. S. (2022). Population fluctuations and synanthropy explain transmission risk in rodent-borne zoonoses. *Nature communications*, *13*(1), 7532.

Ezenwa, V. O., Archie, E. A., Craft, M. E., Hawley, D. M., Martin, L. B., Moore, J., & White, L. (2016). Host behaviour–parasite feedback: an essential link between animal behaviour and disease ecology. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1828), 20153078.

Godó, L., Valkó, O., Borza, S., & Deák, B. (2022). A global review on the role of small rodents and lagomorphs (clade Glires) in seed dispersal and plant establishment. *Global Ecology and Conservation*, *33*, e01982.

Habig, B., & Archie, E. A. (2015). Social status, immune response and parasitism in males: a meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1669), 20140109.

Harrison, A., Scantlebury, M., & Montgomery, W. I. (2010). Body mass and sex-biased parasitism in wood mice *Apodemus sylvaticus*. *Oikos*, *119*(7), 1099-1104.

Jędrzejewski, W., & Jędrzejewska, B. (1993). Predation on rodents in Białowieza primeval forest, Poland. *Ecography*, *16*(1), 47-64.

Krasnov, B. R., Bordes, F., Khokhlova, I. S., & Morand, S. (2012). Gender-biased parasitism in small mammals: patterns, mechanisms, consequences. *Mammalia* 76(1), 1-13.

Moore, J. (2002). *Parasites and the behavior of animals*. Oxford University Press.

Morand, S., De Bellocq, J. G., Stanko, M., & Miklisová, Y. D. (2004). Is sex-biased ectoparasitism related to sexual size dimorphism in small mammals of Central Europe?. *Parasitology*, *129*(4), 505-510.

Sih, A., Bell, A., & Johnson, J. C. (2004). Behavioral syndromes: an ecological and evolutionary overview. *Trends in ecology & evolution*, *19*(7), 372-378.

Sih, A., Spiegel, O., Godfrey, S., Leu, S., & Bull, C. M. (2018). Integrating social networks, animal personalities, movement ecology and parasites: a framework with examples from a lizard. *Animal behaviour*, *136*, 195-205.

Stephens, R. B., & Rowe, R. J. (2020). The underappreciated role of rodent generalists in fungal spore dispersal networks. *Ecology*, *101*(4), e02972.

Zhang, Y., Zhang, Z., & Liu, J. (2003). Burrowing rodents as ecosystem engineers: the ecology and management of plateau zokors *Myospalax fontanierii* in alpine meadow ecosystems on the Tibetan Plateau. *Mammal Review*, *33*(3-4), 284-294.

CHAPTER I

HOST BODY MASS, NOT SEX, AFFECTS ECTOPARASITE LOADS IN YELLOW-NECKED MOUSE (*APODEMUS FLAVICOLLIS*)

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RESEARCH

Host body mass, not sex, affects ectoparasite loads in yellow-necked mouse *Apodemus flavicollis*

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Abstract

We investigated the presence and potential causes of sex bias in ectoparasite infestations in the yellow-necked mouse *Apodemus flavicollis*. We compared the natural tick and flea burdens of male and female mice in a temperate beech forest and assessed whether the observed differences were driven by host sex or body mass. We found that males were more heavily infested by ticks compared to female mice. However, this difference was driven by host body mass, and not sex itself. Host body mass positively correlated with flea loads, but there was no evidence of sex bias in flea abundance. In addition, the abundance of both ticks and fleas infesting yellow-necked mice changed over time, both seasonally (month to month) and annually (year to year). Our results underscore the importance of the sexual size dimorphism and the parasite taxon as the primary factors that influence the occurrence of sex-biased parasitism in small mammals.

Keywords Sex-biased parasitism · Ectoparasites · *Ixodes ricinus* · Siphonaptera · *Apodemus* · Small mammals

Introduction

Sex-biased parasitism has been observed in numerous bird and mammal species (Zuk and McKean 1996; Poulin 1996; Schalk and Forbes 1997; Morand and Poulin 1998; Klein 2000; Wilson et al. 2002; Krasnov et al. 2012). However, the mechanisms behind this pattern remain a subject of debate, primarily because identifying the driving factor can be difficult if it is correlated with other unrelated traits (Zuur et al. 2010; Dormann et al. 2013). Moreover, parasite populations are affected by a number of factors, such as host population density, habitat selection, social or reproductive strategies, and behavioral types, that can interact with host gender (Klein 2004; Gutowsky et al. 2015; Wat et al. 2020). Consequently, unraveling the mechanisms behind sex-biased parasitism has proven to be a challenging task.

The sexual size dimorphism is among the factors that can affect parasite loads. Host body size and condition can impose constraints on the growth and composition of the parasite communities because they represent both the resource and the habitat for parasite populations (Brailsford and Mapes 1987; Price 1990; Christe et al 2003; Krasnov et al. 2005a, b; Bourgoin et al. 2021). As a consequence of intrasexual competition and the action of sex hormones, males are larger than females in most species of mammals (Weckerly 1998; Badyaev 2002; Isaac 2005). They are also frequently more parasitized than females (Schalk and Forbes 1997; Krasnov et al. 2012). Thus, it can be challenging to conclude whether parasites preferably infest males or simply choose larger individuals, who often happen to be males.

Another well-known explanation for sex-biased parasitism is the higher immunocompetence observed in females. This phenomenon is common among many vertebrate taxa (Zuk and McKean 1996; Poulin 1996; Waterman et al. 2013) and is associated with the action of sex hormones: estrogens stimulate immunity while androgens depress it (Folstad and Karter 1992; Schalk and Forbes 1997; Klein 2000). Steroid sex hormones may also affect resistance to diseases by altering the expression of major histocompatibility complex (MHC) genes (Klein 2000). Since immunity is a crucial defense mechanism

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against parasite infections, sexual hormones may indirectly affect the richness and abundance of parasite communities harbored by male and female hosts.

Finally, the life cycles and biology of parasites can also shape their interaction with the host. This relationship can also vary, depending on environmental conditions (Leung and Poulin 2008). For some parasites, it may be easier or more advantageous to inhabit males than females, depending on the sex-specific behavioral or physiological traits of the host. Additionally, as male and female hosts can interact differently with their environment, varying habitat qualities can also affect parasite transmission in a sex-specific manner. Therefore, it is essential to consider how parasite and habitat-specific traits mediate the interactions between male and female hosts and their parasites.

The purpose of this study was to compare the ectoparasite burden of male and female yellow-necked mice (*Apodemus flavicollis*) and to determine whether any potential gender bias is driven by the sex or body mass of the host. Our research is based on a similar project by Harrison et al. (2010), where authors estimated natural tick loads of wild wood mouse (*Apodemus sylvaticus*) populations in Irish mixed broadleaf and coniferous forest. Their results suggested that differences in parasite burdens between males and females were due to sex-related differences in body mass, not the sex itself. In this study, we follow their methodology and conduct analogical analyses to test if similar patterns occur in a congeneric rodent, *A. flavicollis,* in a temperate beech forest in Poland. As such, this study is a quasi-replication (Nakagawa and Parker 2015; Palmer 2000) of the research conducted by Harrison and colleagues (2010). Furthermore, we tested whether similar patterns are found in flea infestations of *A. flavicollis*. Our study species exhibits sexual size dimorphism (Schulte-Hostedde 2007), therefore we expected male-biased parasite burdens and predicted that both tick and flea numbers would be higher in males due to their greater body mass, not because of their sex. Our specific questions were as follows:

i. Do males carry higher ectoparasite loads than females?
ii. Does the sex bias in ectoparasite infestation persist after accounting for host sexual dimorphism?
iii. What is the relationship between male body mass and ectoparasite loads?
iv. What is the relationship between female body mass and ectoparasite loads?
v. Do the above relationships differ for ticks and fleas?

Materials and methods

Study site

This study took place in Forest Inspectorate Łopuchówko, Buczyna district, located in Greater Poland Voivodeship, N-W Poland. The maximum altitude at the study site is 143 m above sea level and the landscape is mostly flat or hilly. The temperatures range from an average of -2.5 °C in January to 18.2 °C in July, and the annual precipitation averages 520 mm. The study sites were situated in managed forests, primarily consisting of European beech (*Fagus sylvatica),* along with other species, such as pedunculate oak (*Quercus robur)*, red oak (*Quercus rubra)*, European hornbeam (*Carpinus betulus),* and sycamore maple (*Acer pseudoplatanus)* (categorized as habitat 9130, 'Asperulo-Fagetum' according to the EU Habitat Directive).

Small mammal live-trapping and ectoparasite sampling

We established six trapping grids, each with 100 live traps, arranged in a 10 × 10 pattern, with 10-m spacing between the traps. To minimize the movement of mice between the grids, each grid was located at least 300 m apart. Trapping was carried out during three summer seasons (July–August 2018–2020). One trapping session consisted of four or five nights per site, and we conducted three (2018–19) or five (2020) trapping sessions per site. The total trapping effort amounted to 30,000 trap-nights (9,000 in 2018 and 2019, and 12,000 in 2020).

At the first capture, all animals were assigned to species and marked with unique aluminum ear tags (National Band and Tag Company, mouse tags type 1005–1). We recorded the body mass of all individuals at each capture using the PESOLA scale (0.5 g accuracy), and visually determined their sex and reproductive status (scrotal or non-scrotal males, lactating, pregnant or nonpregnant females, and juveniles of both sexes). Shrews (*Sorex araneus* and *S. minutus*) were released unmarked.

After recording data on body mass and reproductive condition, we collected all fleas found on the host and in the handling bag. We then counted all ticks attached to the host, which were primarily located on the head and ears, though we searched the entire body. A random subset of 20 ticks was collected from each mouse to identify the tick species (fleas were not identified to species in this study) using laboratory molecular methods. Total genomic DNA was extracted from each tick individually using the ammonium hydroxide method (Rijpkema and Bruinink 1996). The tick species were determined using sequence data from the fragment of the cytochrome *c* oxidase subunit I (COI). The material was sequenced using Ion Torrent S5 System (Thermo Fisher, USA) and the results were compared with GenBank reference sequences.

Statistical analysis

All statistical analyses were performed with R in RStudio IDE (R Core Team 2018; RStudio Team 2020). We used generalized linear mixed models (GLMMs, Bolker et al. 2009) implemented via the glmmTMB package (Brooks et al. 2017; Magnusson et al. 2017) and assessed fit with DHARMa and performance packages (Hartig and Hartig 2017; Lüdecke et al. 2021). To separate the influence of gender and body mass on ectoparasite burdens, we followed the statistical approach used by Harrison et al. (2010) with these adjustments:

a) We fitted our models to both tick and flea data.
b) We used the negative binomial error distribution with a log-link function.
c) We included additive effects of month and year effects to control for seasonal and year-to-year changes in ectoparasite numbers (Langley and Fairley 1982; Gray 1991; Herrero-Cófreces et al. 2021).
d) We adjusted the structure of the tick models to zero-inflated count data to account for the excess of zeros.
e) To account for the nested data structure (Schielzeth and Nakagawa 2013), we included random effects of an individual mouse and trapping site.

Because pregnancy can confound the relationship between body mass, sex, and parasitism (Harrison et al. 2010), we excluded pregnant mice from the data set. We assessed pregnancy based on two traits: i) visibly enlarged belly, and ii) increased body mass compared to other trappings of the same individual. We also excluded juveniles from the data, we based our selection on body mass because we found pelage color to be overly subjective. We chose 15 g of body mass as a cut-off value between juveniles and adults (Pucek et al. 1993). However, the growth rate and the onset of reproduction in the yellow-necked mouse vary with food availability and other environmental factors (Gliwicz 1988; Balčiauskienė et al. 2009; Sawicka-Kapusta 1968; Ferrari et al. 2004), therefore, we explored the sensitivity of our results to different values of this threshold.

To address our first question (i. whether males carry higher ectoparasite loads), we fitted “model 1”, which tested the influence of host sex on tick load without considering the effect of host body mass. To address question two (ii. whether there is a sex bias in tick and flea loads after controlling for the effect of body mass), we paired males and females with equal weight. If an exact match was impossible, we paired individuals with a difference of no more than 0.5 g. No mouse was paired twice within one trapping session, but we allowed the same individual to be paired again in other sessions. We created analogical datasets for both ticks and fleas. We ran the paired model (“model 2”) using host sex as explanatory variable, with the pair ID as random effect. To tackle questions iii and iv – are heavier males/females more parasitized? – we divided the dataset into males and females and ran two models:”model 3″ to check the effect of body mass within the male sex, and “model 4″ to assess the effect of body mass within the female sex (the numeration of models follows Harrison et al. 2010). To address question v. we compared the effect sizes of the models fitted for tick and flea data with results obtained by Harrison et al. 2010 and other similar studies.

Results

The small mammal community at our study sites was dominated by *Apodemus flavicollis* (66.9% of captured individuals) and bank vole (*Myodes glareolus*) (31.7% of individuals), followed by other species such as common vole (*Microtus arvalis*) *common shrew* (*Sorex araneus*), *Eurasian pygmy shrew* (*S. minutus*), and striped field mouse (*Apodemus agrarius*). In total, we captured 1873 unique mice, 200 in 2018 (116 males, 80 females, 4 unassessed), 880 in 2019 (414 males, 465 females, 1 unassessed), and 793 in 2020 (421 males, 370 females, 2 unassessed). The average body mass was higher in male compared to female yellow-necked mice (30.6 g vs. 26.0 g, t = -21.471, 95% CI: -5.07 – -4.22, df = 5732.9, P < 0.001).

Effect of sex and body mass on tick loads

Overall (including recaptures), we sampled ticks 2079 times: 1065 times from male hosts and 1014 times from females. *Ixodes ricinus* was the only tick species found. According to model 1, that is without accounting for body mass, males carry more ticks on average than females (males 16.1 ticks, 95% CI: 14.3 – 18.1 vs. females: 12.9 ticks, 95% CI: 11.4 – 14.6; z = 4.95, P = 0.001). After controlling for the effect of body mass (model 2 that paired males and females of the same weight), the sex bias did not longer persist (z = 1.484, P = 0.138). The two models (3 and 4) in which we tested both sexes separately detected a positive association of body mass with tick loads in both males and females (males: z = 6.305, P < 0.001, N = 663; females: z = 3.757, P < 0.001, N = 561; Fig. 1). The effect of month and year was significant in every model (Supplementary Table 1, Supplementary Materials Fig. 1).

Effect of sex and body mass on flea burdens

Overall (including recaptures), we sampled fleas 2010 times: 1023 times from males and 987 times from females. In contrast to ticks, Model 1 did not reveal a sex bias in flea infestation (z = 0.97, P = 0.332). Similarly, Model 2 (with males and females paired by mass) did not find the effect of sex (z = -1.146, P = 0.271). However, when the sexes were tested separately (models 3 and 4), body mass was positively associated with both male (z = 3.230, p = 0.001) and female

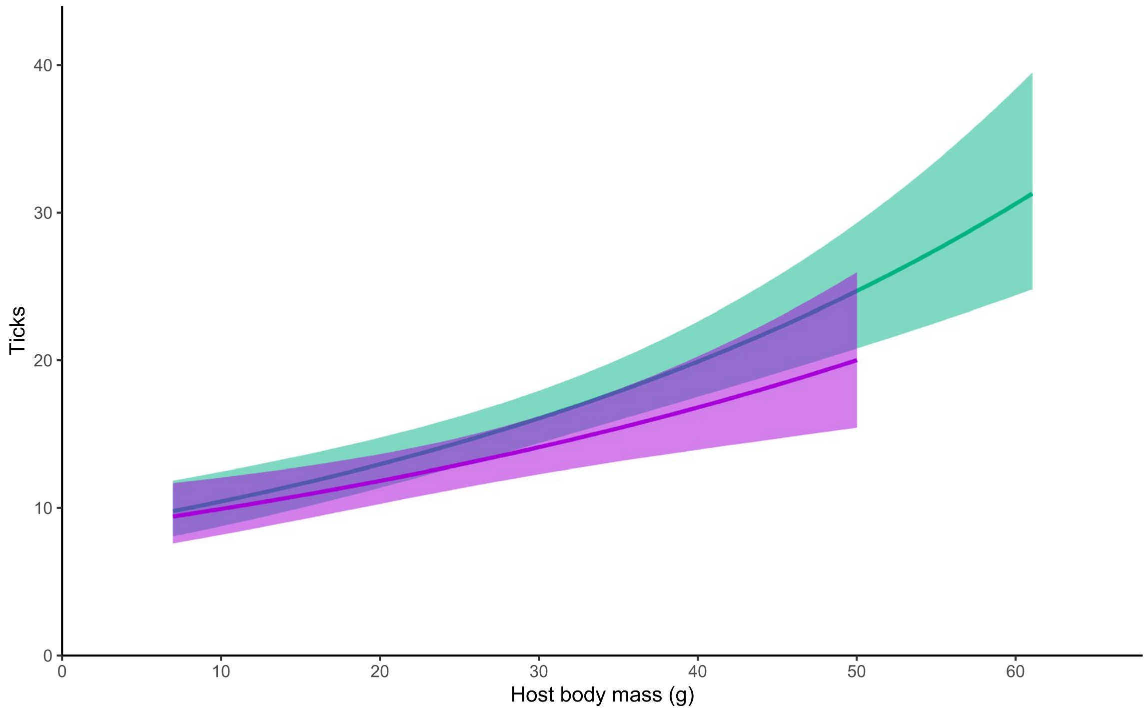

Fig. 1 Estimated tick abundance in male (green) and female (violet) *A. flavicollis*. Shading corresponds to a 95% confidence interval. See Table 1, models 3 and 4, for more details

Table 1 Factors influencing the abundance of ticks infesting yellow-necked mice (*A. flavicollis*). Model (1) estimates the effect of sex without controlling for body mass, model (2) controls for body mass by pairing males with females of the same mass, model (3) estimates the effect of body mass on tick abundance in male hosts, and model (4) does the same for females. All models controlled the effect of month and year. Juveniles were excluded at the 15 g threshold. Random effects always included individual host and trapping site

Model	Effect	N	β ± SE	P
1	Sex	1224	0.216 ± 0.044	< 0.001***
2	Sex (paired)	481	0.080 ± 0.054	0.138
3	Body mass	663 (only males)	0.021 ± 0.003	< 0.001***
4	Body mass	561 (only females)	0.017 ± 0.005	< 0.001***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

flea loads (z = 3.640, P < 0.001; see Fig. 2). We observed a significantly higher number of fleas in 2018, and flea abundance decreased in August compared to June and July (Supplementary Table 2, Supplementary Fig. 2).

Discussion

We observed a male bias in tick loads of yellow-necked mice. However, when we accounted for differences in body mass, this pattern no longer persisted. This result indicates that sex-biased parasitism in this system is driven primarily by body mass, rather than other sex-related traits. In the case of flea abundance, we did not find any sex-related effects: both males and females carried similar flea loads, even when accounting for body mass. Only the host's body mass had a significant impact on flea loads.

The study we here quasi-replicated (Harrison et al. 2010, Fig. 3) also had found male mice to carry more ticks and concluded that this pattern could be related to sexual size dimorphism. Our study, conducted on a different rodent species (*A. flavicollis* rather than *A. sylvaticus*), in a different geographic location (Poland vs. Ireland), in a different forest type (beech vs. mixed broadleaf and coniferous), and with a considerably larger sample size (1214 vs. 288 mice), produced similar findings. This convergence of results indicates that the relationship between *I. ricinus* and its hosts *Apodemus* spp. is robust. Our study showed a similarity in the pattern of sex bias in tick burdens in *Apodemus* spp. between Ireland and Poland when comparing the effect sizes of both studies (Fig. 3). However, the effect sizes observed in our study were consistently smaller than those in the original study. A comparison of effect sizes of both studies demonstrated that sex bias in tick burdens in *Apodemus* spp. followed a similar pattern in both Ireland and Poland. On the other hand, the effect sizes that we detected were consistently smaller than the ones from the original study. In particular, the effect of sex on tick loads, while significant, was weaker in our study.

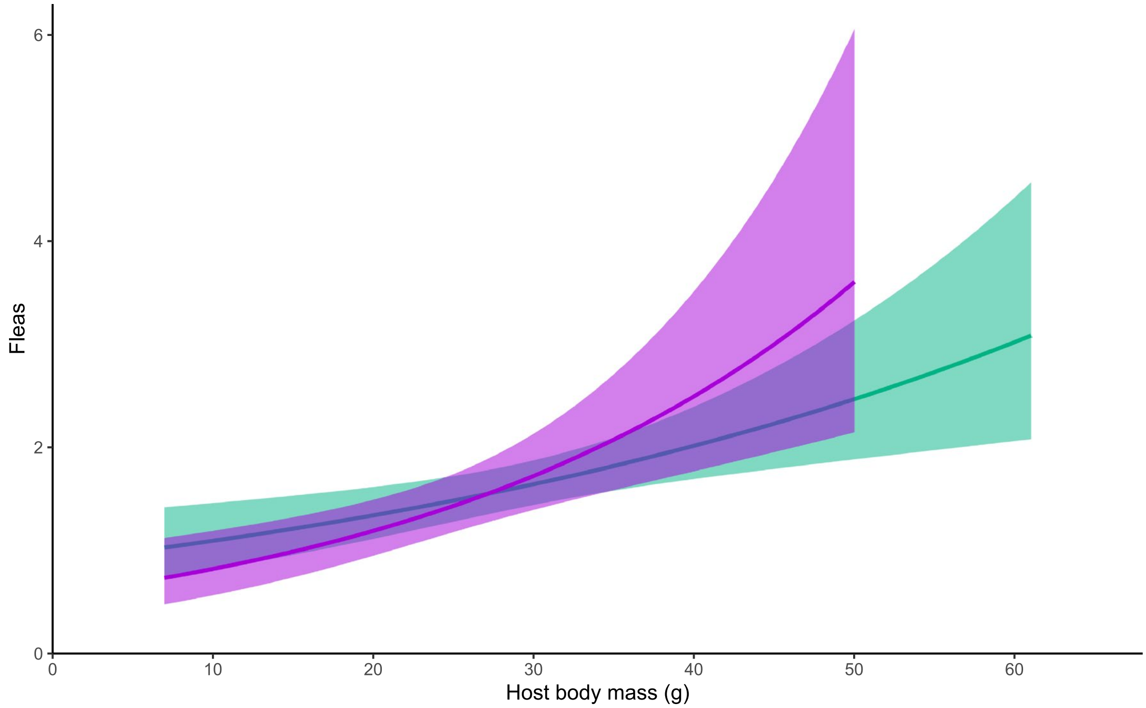

Fig. 2 Estimated flea abundance in male (green) and female (violet) *A. flavicollis*. Shading corresponds to a 95% confidence interval. See Table 2 (models 3 and 4) for more details

Table 2 Factors influencing the abundance of fleas infesting yellow-necked mice (*Apodemus flavicollis).* Model (1) estimates the effect of sex without taking into account the body mass of the host, model (2) controls for body mass by pairing males with females of the same mass, model (3) estimates the effect of body mass on flea numbers harbored by male hosts, and model (4) does the same for females. All models controlled the effect of month and year. Juveniles were excluded at the 15 g threshold. Random effects always included individual host and trapping site

Model	Effect	N	β ± SE	P
1	Sex	1224	0.082 ± 0.085	0.332
2	Sex (paired)	481	-0.146 ± 0.133	0.272
3	Body mass	663 (only males)	0.020 ± 0.006	0.001**
4	Body mass	561 (only females)	0.037 ± 0.010	< 0.001***

$*p < 0.05$; $**p < 0.01$; $***p < 0.001$

In contrast to ticks, there was no evidence of male bias in flea parasitism (models 1 and 2). Similar results were reported by other authors (Benedek and Sirbu 2016; Kowalski et al. 2015). Interestingly, our models 3 and 4 demonstrated that that both males and females alike, heavier individuals carried higher loads of fleas (a pattern not detected in Kowalski et al. 2015, perhaps because of their smaller sample size). The most likely explanation for this pattern is that, even though flea abundance is affected by body size, and body size is influenced by sex, these effects were not strong enough to generate a clear-cut difference in flea infestation between males and females.

The positive effect of body mass on tick and flea infestation that we observed might be caused by several factors. Firstly, larger-bodied hosts could be easier targets to find and colonize (Hawlena et al. 2005; Harrison et al. 2010; Kiffner et al. 2013), which is especially relevant for parasites that actively seek their hosts, such as ticks. Secondly, if a larger resource patch can sustain more inhabitants, bigger hosts should have a higher parasitic burden (Presley and Willig 2008). Bigger host can also favor coexistence among parasites by reducing both intra- and inter-specific competition, providing a greater variety of accessible niches and better resource division (Kuriset al. 1980; Gregory et al. 1996; Morand and Poulin 1998; Kiffner et al. 2013). Finally, it could be more difficult for smaller hosts to tolerate a high ectoparasitic burden. This could lead to size-dependent differences in grooming, which would result in lower numbers of ectoparasites in small-bodied hosts (Hart et al. 1992; Hawlena et al. 2008). Self-grooming is a time-consuming activity that may be less critical for larger individuals, as they can access resources such as food and mates more easily and are often in better body condition, which allows them to compensate for the energy lost due to parasite infestation. Furthermore, the energy loss caused by parasites is relatively less significant for larger individuals than for smaller ones,

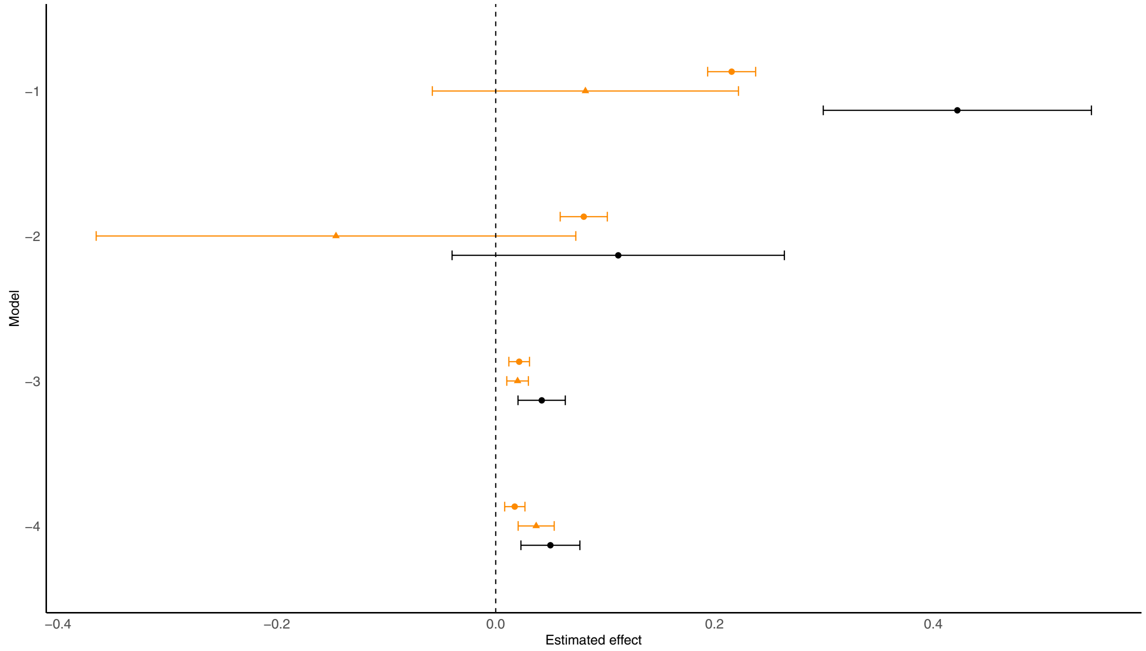

Fig. 3 Estimated effect of sex (model 1 and 2) and body mass (models 3 and 4) of *A. flavicollis* (yellow, this study) and *A. sylvaticus* (black, Harrison et al. 2010) on their tick (circles) and flea (triangles) burdens. Error bars correspond to 95% confidence intervals. See Tables 1 and 2 for more information on the models

making it more viable for larger hosts to neglect thorough cleaning of their fur. A larger body requires longer bouts of self-grooming to keep the parasite population at bay. Therefore, larger animals are either forced to spend proportionately more time on self-grooming or tolerate relatively higher parasite loads to engage in other vital activities such as foraging or reproduction (Raveh et al. 2011).

Furthermore, body mass is linked with other traits that may affect parasite acquisition. In several rodent species, male body mass has been demonstrated to have a positive correlation with home range size (Borowski 2003). Defending a larger home range requires increased mobility and social interactions, which can lead to a heightened risk of parasitism (Gregory et al. 1996; Jetz et al. 2004; Kiffner et al. 2014). In addition, higher testosterone levels in males are associated with greater body mass and testes size, causing behavioral changes that elevate the risk of parasite transmission through fights with competitors and mating (Forbes 1985; Royland et al. 1994; Breed and Taylor 2000).

In contrast, female mice tend to be less mobile and have smaller home ranges (Bergstedt 1966; Attuquayefio et al. 1986; Stradiotto et al. 2009). Additionally, female hormones such as estrogen are believed to have an immunostimulating effect, unlike testosterone (Klein 2004). On the other hand, females tend to have more social interactions than males, staying closer to the natal site after the juvenile stage and residing in nests with their offspring to provide parental care (Wolff 2007). Aggregation is considered a risk factor for parasitism, as it intensifies transmission rates (Anderson & May 1979; May and Anderson 1979; Arneberg et al. 1998; Krasnov et al. 2002; Christe et al. 2007).

Our findings highlight the importance of the sexual size dimorphism in shaping sex-biased parasitism patterns among small mammals (Moore and Wilson 2002; Harrison et al. 2010; Kowalski et al. 2015; Merabet et al. 2021, but see Morand et al. 2004; Krasnov et al. 2005a, b; Perez-Orella and Schulte-Hostedde 2005; Gorrell Jamieson and Schulte-Hostedde 2008). The mechanisms driving sex-biased parasitism can be intricate and involve interactions between various host and parasite traits, as well as environmental factors. Nevertheless, our study's results are consistent with a similar study on a congeneric rodent species conducted in a different geographical location, which aids in generalizing tick parasitism patterns. The sex bias in flea infestations of *Apodemus* spp. appears to be less clear and might depend on the host species (Morand et al. 2004; Kiffner et al. 2013; Kowalski et al. 2015). While numerous studies have reported a link between ectoparasite infestation and host body size, the relationship is not consistently demonstrated and might vary across study systems and is not consistently demonstrated (Perez-Orella and Schulte-Hostedde 2005; Krasnov et al. 2011; 2012; Kiffner et al. 2014; Herrero-Cófreces et al. 2021). This varying relationship between ectoparasite infestation and host body size has broad implications for both host and parasite ecology and evolution, as well as epidemiological applications for control of zoonotic infections.

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Authors contributions SS, MZ, RZ conceived and designed the study. MZ, SS, AW collected the data. SS, MZ, AW processed and prepared data for analysis. SS, RZ and MZ analyzed the data. SS and MZ wrote the manuscript. SS prepared Fig. 1 and 2, MZ prepared Fig. 3 and Supplementary Materials. AW and RZ provided critical feedback and helped shape the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets used for analyses are available from the corresponding author upon request.

Declarations

Ethics approval This study was conducted in accordance with the guidelines of the Polish Society for Laboratory Animal Science (PolLASA). The Local Ethical Committee in Poznań approved all experimental procedures (Approval No. 24/2018).

Consent for publication We declare that all authors have read and approved the manuscript for submission to Parasitology Research. We affirm that the manuscript is original and has not been published previously, nor is it currently under consideration for publication elsewhere.

Competing interests The authors declare that they have no competing interests of financial nor personal nature.

References

Anderson RM, May RM (1979) Population biology of infectious diseases: Part I. Nature 280(5721):361–367. https://doi.org/10.1038/280361a0

Arneberg P, Skorping A, Grenfell B, Read AF (1998) Host densities as determinants of abundance in parasite communities. Proc R Soc Lond b: Biol Sci 265:1283–1289. https://doi.org/10.1098/rspb.1998.0431

Attuquayefio DK, Gorman ML, Wolton RJ (1986) Home range sizes in the wood mouse Apodemus sylvaticus: habitat, sex and seasonal differences. J Zool 210:45–53. https://doi.org/10.1111/j.1469-7998.1986.tb03619.x

Badyaev AV (2002) Growing apart: an ontogenetic perspective on the evolution of sexual size dimorphism. Trends Ecol Evol 17:369–378. https://doi.org/10.1016/S0169-5347(02)02569-7

Balčiauskienė L, Balčiauskas L, Čepukienė A (2009) Demographic and morphometric parameters of the yellow-necked mouse (Apodemus flavicollis) in late autumn-early spring in Lithuania. Acta Biol Univ Daugavp 9:25–34

Bergstedt B (1966) Home ranges and movements of the rodent species Clethrionomys glareolus (Schreber), Apodemus flavicollis (Melchior) and Apodemus sylvaticus (Linné) in southern Sweden. Oikos 150–157. https://doi.org/10.2307/3564939

Benedek AM, Sirbu I (2016) Influence of habitat, time and host variables on prevalence of parasitic arthropods in bank vole, Myodes glareolus (Schreber, 1780) in Transylvania (Romania), Brukenthal. Acta Musei, XI. 3

Bolker BM, Brooks ME, Clark CJ, Geange SW et al (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evol 24:127–135. https://doi.org/10.1016/j.tree.2008.10.008

Borowski Z (2003) Habitat selection and home range size of field voles Microtus agrestis in Słowiński National Park, Poland. Acta Theriol 48:325–333. https://doi.org/10.1007/BF03194172

Bourgoin G, Portanier E, Poirel M, Itty C, Duhayer J, Benabed S, Garel M (2021) Reproductive females and young mouflon (Ovis gmelini musimon × Ovis sp.) in poor body condition are the main spreaders of gastrointestinal parasites. Parasitology 148:809–818. https://doi.org/10.1017/S0031182021000329

Brailsford T, Mapes C (1987) Comparisons of Heligmosomoides polygyrus primary infection in protein-deficient and well-nourished mice. Parasitology 95:311–321. https://doi.org/10.1017/S0031182000057760

Breed WG, Taylor J (2000) Body mass, testes mass, and sperm size in murine rodents. J Mammal 81:758–768. https://doi.org/10.1644/1545-1542(2000)081%3c0758:BMTMAS%3e2.3.CO;2

Brooks ME, Kristensen K, Van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Machler M, Bolker BM (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R journal 9(2):378–400

Christe P, Giorgi MS, Vogel P, Arlettaz R (2003) Differential species-specific ectoparasitic mite intensities in two intimately coexisting sibling bat species: resource-mediated host attractiveness or parasite specialization? J Anim Ecol 72:866–872. https://doi.org/10.1046/j.1365-2656.2003.00759.x

Christe P, Glaizot O, Evanno G, Bruyndonck N et al (2007) Host sex and ectoparasites choice: preference for, and higher survival on female hosts. J Anim Ecol 76:703–710. https://doi.org/10.1111/j.1365-2656.2007.01255.x

Dormann CF, Elith J, Bacher S, Buchmann C, Carl G, Carré G et al (2013) Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography 36:27–46. https://doi.org/10.1111/j.1600-0587.2012.07348.x

Ferrari N, Cattadori IM, Nespereira J, Rizzoli A, Hudson PJ (2004) The role of host sex in parasite dynamics: field experiments on the yellow-necked mouse Apodemus flavicollis. Ecol Lett 7:88–94. https://doi.org/10.1046/j.1461-0248.2003.00552.x

Folstad I, Karter AJ (1992) Parasites, bright males, and the immunocompetence handicap. Am Nat 139:603–622. https://doi.org/10.1086/285346

Forbes GB (1985) The effect of anabolic steroids on lean body mass: the dose response curve. Metabolism 34:571–573. https://doi.org/10.1016/0026-0495(85)90196-9

Gliwicz J (1988) Seasonal dispersal in non-cyclic populations of Clethrionomys glareolus and Apodemus flavicollis. Acta Theriol 33:263–272

Gorrell Jamieson C, Schulte-Hostedde AI (2008) Patterns of parasitism and body size in red squirrels (Tamiasciurus hudsonicus). Can J Zool 86:99–107. https://doi.org/10.1139/z07-123

Gray JS (1991) The development and seasonal activity of the tick *Ixodes ricinus*: a vector of Lyme borreliosis. Rev Med Vet Entomol 79(6):323–333

Gregory RD, Keymer AE, Harvey PH (1996) Helminth parasite richness among vertebrates. Biodivers Conserv 5:985–997. https://doi.org/10.1007/BF00054416

Gutowsky LFG, Harrison PM, Martins EG, Leake A, Patterson DA, Cooke SJ (2015) Interactive effects of sex and body size on the movement ecology of adfluvial bull trout (Salvelinus confluentus). Can J Zool 94:31–40. https://doi.org/10.1139/cjz-2015-0104

Harrison A, Scantlebury M, Montgomery WI (2010) Body mass and sex-biased parasitism in wood mice Apodemus sylvaticus. Oikos 119:1099–1104. https://doi.org/10.1111/j.1600-0706.2009.18072.x

Hart BL, Hart LA, Mooring MS, Olubayo R (1992) Biological basis of grooming behavior in antelope: the body-size, vigilance, and habitat principles. Anim Behav 44:615–631. https://doi.org/10.1016/S0003-3472(05)80290-8

Hartig F, Hartig MF (2017) Package 'DHARMa'. R Package Version

Hawlena H, Abramsky Z, Krasnov BR (2005) Age-biased parasitism and density-dependent distribution of fleas (Siphonaptera) on a desert rodent. Oecologia 146:200–208. https://doi.org/10.1007/s00442-005-0187-0

Hawlena H, Bashary D, Abramsky Z, Khokhlova IS et al (2008) Programmed versus stimulus-driven antiparasitic grooming in a desert rodent. Behav Ecol 19:929–935. https://doi.org/10.1093/beheco/arn046

Herrero-Cófreces S, Flechoso MF, Rodríguez-Pastor R, Luque-Larena JJ, Mougeot F (2021) Patterns of flea infestation in rodents and insectivores from intensified agro-ecosystems, Northwest Spain. Parasite Vector 14:16. https://doi.org/10.1186/s13071-020-04492-6

Isaac JL (2005) Potential causes and life-history consequences of sexual size dimorphism in mammals. Mammal Rev 35:101–115. https://doi.org/10.1111/j.1365-2907.2005.00045.x

Jetz W, Carbone C, Fulford J, Brown JH (2004) The scaling of animal space use. Science 306:266–268. https://doi.org/10.1126/science.11021

Kiffner C, Stanko M, Morand S, Khokhlova IS et al (2013) Sex-biased parasitism is not universal: evidence from rodent–flea associations from three biomes. Oecologia 173:1009–1022. https://doi.org/10.1007/s00442-013-2664-1

Kiffner C, Stanko M, Morand S, Khokhlova IS et al (2014) Variable effects of host characteristics on species richness of flea infracommunities in rodents from three continents. Parasitol Res 113:2777–2788. https://doi.org/10.1007/s00436-014-3937-2

Klein SL (2000) The effects of hormones on sex differences in infection: from genes to behavior. Neuroscience 24:627–638. https://doi.org/10.1016/S0149-7634(00)00027-0

Klein SL (2004) Hormonal and immunological mechanisms mediating sex differences in parasite infection. Parasite Immunol 26:247–264. https://doi.org/10.1111/j.0141-9838.2004.00710.x

Kowalski K, Bogdziewicz M, Eichert U, Rychlik L (2015) Sex differences in flea infections among rodent hosts: is there a male bias? Parasitol Res 114:337–341. https://doi.org/10.1007/s00436-014-4231-z

Krasnov BR, Khokhlova I, Shenbrot G (2002) The effect of host density on ectoparasite distribution: an example of a rodent parasitized by fleas. Ecology 83:164–175. https://doi.org/10.1890/0012-9658(2002)083[0164:TEOHDO]2.0.CO;2

Krasnov BR, Morand S, Hawlena H, Khokhlova IS et al (2005a) Sex-biased parasitism, seasonality and sexual size dimorphism in desert rodents. Oecologia 146:209–217. https://doi.org/10.1007/s00442-005-0189-y

Krasnov BR, Khokhlova IS, Arakelyan MS, Degen AA (2005b) Is a starving host tastier? Reproduction in fleas parasitizing food-limited rodents. Fun Ecol 19:625–631. https://doi.org/10.1111/j.1365-2435.2005.01015.x

Krasnov BR, Stanko M, Matthee S, Laudisot A, Leirs H, Khokhlova IS, Korallo-Vinarskaya NP, Vinarski MV, Morand S (2011) Male hosts drive infracommunity structure of ectoparasites. Oecologia 166:1099–1010. https://doi.org/10.1007/s00442-011-1950-z

Krasnov BR, Bordes F, Khokhlova IS, Morand S (2012) Gender-biased parasitism in small mammals: patterns, mechanisms, consequences. Mammalia 76:1–13. https://doi.org/10.1515/mammalia-2011-0108

Kuris AM, Blaustein AR, Alio JJ (1980) Hosts as islands. Am Nat 116:570–586. https://doi.org/10.1086/283647

Langley R, Fairley JS (1982) Seasonal variations in infestations of parasites in a Wood mouse *Apodemus sylvaticus* population in the West of Ireland. J Zool 198:249–261. https://doi.org/10.1111/j.1469-7998.1982.tb02074.x

Leung TL, Poulin R (2008) Parasitism, commensalism, and mutualism: exploring the many shades of symbioses. Vie et Milieu/Life & Environment 58(2):107–115

Lüdecke D, Ben-Shachar MS, Patil I, Waggoner P et al (2021) performance: An R package for assessment, comparison and testing of statistical models. J Open Source Software 6(60):3139. https://doi.org/10.21105/joss.03139

Magnusson A, Skaug H, Nielsen A, Berg C et al (2017) Package 'glmmTMB'. R Package Version 0.2.0.

May RM, Anderson RM (1979) Population biology of infectious diseases: Part II. Nature 280(5722):455–461. https://doi.org/10.1038/280455a0

Merabet S, Khammes-El Homsi N, Aftisse L, Khammes-Talbi N, Milla A, Morand S, Ribas A (2021) Helminth parasites in the wood mouse (Apodemus sylvaticus) from Algeria. Arxius de Miscellània Zoològica 19:205–212. https://doi.org/10.32800/amz.2021.19.0205

Morand S, Poulin R (1998) Density, body mass and parasite species richness of terrestrial mammals. Evol Ecol 12:717–727. https://doi.org/10.1023/A:1006537600093

Morand S, De Bellocq JG, Stanko M, Miklisová YD (2004) Is sex-biased ectoparasitism related to sexual size dimorphism in small mammals of Central Europe? Parasitology 129:505–510. https://doi.org/10.1017/S0031182004005840

Nakagawa S, Parker TH (2015) Replicating research in ecology and evolution: feasibility, incentives, and the cost-benefit conundrum. BMC Biol 13:88. https://doi.org/10.1186/s12915-015-0196-3

Palmer AR (2000) Quasi-replication and the contract of error: lessons from sex ratios, heritabilities and fluctuating asymmetry. Annu Rev Ecol Evol Syst 31:441–480. https://doi.org/10.1146/annurev.ecolsys.31.1.441

Perez-Orella C, Schulte-Hostedde AI (2005) Effects of sex and body size on ectoparasite loads in the northern flying squirrel (Glaucomys sabrinus). Can J Zool 83:1381–1385. https://doi.org/10.1139/z05-126

Poulin R (1996) Sexual inequalities in helminth infections: a cost of being a male? Am Nat 147:287–295. https://doi.org/10.1086/285851

Presley SJ, Willig MR (2008) Intraspecific patterns of ectoparasite abundances on Paraguayan bats: effects of host sex and body size. J Trop Ecol 24:75–83. https://doi.org/10.1017/S0266467407004683

Price PW (1990) Host populations as resources defining parasite community organization. In: Esch GW, Bush AO, Aho JM (eds) Parasite Communities: Patterns and Processes. Springer, Dordrecht, 21–40. https://doi.org/10.1007/978-94-009-0837-6_2

Pucek Z, Jędrzejewski W, Jędrzejewska B, Pucek M (1993) Rodent population dynamics in a primeval deciduous forest (Białowieża National Park) in relation to weather, seed crop, and predation. Acta Theriol 38:199–232. https://doi.org/10.4098/AT.arch.93-18

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

RStudio RT (2020) Integrated development environment for R (RStudio, PBC, Boston, MA). https://www.R-project.org

Raveh A, Kotler BP, Abramsky Z, Krasnov BR (2011) Driven to distraction: detecting the hidden costs of flea parasitism through foraging behavior in gerbils. Ecol Lett 14:47–51. https://doi.org/10.1111/j.1461-0248.2010.01549.x

Rijpkema S, Bruinink H (1996) Detection of Borrelia burgdorferi sensu lato by PCR in questing Ixodes ricinus larvae from the Dutch North Sea island of Ameland. Exp Appl Acarol 20:381–385. https://doi.org/10.1007/BF00130550

Royland JE, Weber LJ, Fitzpatrick M (1994) Testes size and testosterone levels in a model for weightlessness. Life Sci 54:545–554. https://doi.org/10.1016/0024-3205(94)90005-1

Sawicka-Kapusta K (1968) Annual fat cycle of field mice, Apodemus flavicollis (Melchior, 1834). Acta Theriol 13:329–339

Schalk G, Forbes MR (1997) Male biases in parasitism of mammals: effects of study type, host age, and parasite taxon. Oikos 78:67–74. https://doi.org/10.2307/3545801

Schielzeth H, Nakagawa S (2013) Nested by design: model fitting and interpretation in a mixed model era. Methods Ecol Evol 4(1):14–24

Schulte-Hostedde AI (2007) Sexual size dimorphism in rodents. Rodent societies: an ecological and evolutionary perspective. 115(128):5

Stradiotto A, Cagnacci F, Delahay R, Tioli S, Nieder L, Rizzoli A (2009) Spatial organization of the yellow-necked mouse: Effects of density and resource availability. J Mammal 90:704–714. https://doi.org/10.1644/08-MAMM-A-120R1.1

Wat KK, Herath AP, Rus AI, Banks PB, Mcarthur C (2020) Space use by animals on the urban fringe: interactive effects of sex and personality. Behav Ecol 31:330–339. https://doi.org/10.1093/beheco/arz194

Waterman JM, Macklin GF, Enright C (2013) Sex-biased parasitism in Richardson's ground squirrels (Urocitellus richardsonii) depends on the parasite examined. Can J Zool 92:73–79. https://doi.org/10.1139/cjz-2013-0151

Weckerly FW (1998) Sexual-size dimorphism: influence of mass and mating systems in the most dimorphic mammals. J Mammal 79:33–52. https://doi.org/10.2307/1382840

Wilson K, Bjrnstad ON, Dobson AP, Merler S et al (2002) Heterogeneities in macroparasite infections: patterns and processes. Ecol Wildl Dis 44:6–44

Wolff JO (2007) Social Biology of Rodents. Int Zool 2:193–204. https://doi.org/10.1111/j.1749-4877.2007.00062.x

Zuk M, McKean KA (1996) Sex differences in parasite infections: Patterns and processes. Int J Parasitol 26:1009–1024. https://doi.org/10.1016/S0020-7519(96)00086-0

Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol 1:3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x

CHAPTER II

PERSONALITY, SPACE USE AND ECTOPARASITE LOADS: RISK-TAKING BEHAVIOR DRIVES NEGATIVE FEEDBACK LOOPS BETWEEN YELLOW-NECKED MICE (*APODEMUS FLAVICOLLIS*) AND SHEEP TICKS (*IXODES RICINUS*)

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(unpublished)

INTRODUCTION

Parasitism is a domain that strongly interacts with host behavior on multiple levels, forming an interesting research frontier at the intersection of parasitology, ethology, and ecology. Parasites can influence the viability of their hosts in multiple ways. On the evolutionary scale, in line with the Red Queen hypothesis, parasites can act as selective force, enhancing the development of more effective immune responses or other anti-parasitic strategies (Dawkins & Krebs 1979). At the population level, parasites can serve as top-down regulators, affecting population viability and dynamics (Neuhaus 2003, Gooderham and Schulte-Hostedde 2011). For individual hosts, parasites can reduce reproductive success and survival (Tompkins & Begon, 1999). The relationships between hosts and parasites are also strongly linked to hosts' behavior, and parasites can alter activity patterns of their hosts simply by being present in the environment: to lower the risk of infection, animals may avoid foraging in parasite-contaminated areas, abandon parasitized burrows (Butler & Roper 1996, Reckardt & Kerth 2007), and reduce contacts with infected individuals within their social groups (Poirotte et al. 2017). These consequences of parasitism can have cascading effects across entire ecosystems (Weinstein et al. 2018, Loker & Hofkin, 2022).

One of the most intriguing aspects of host-parasite interactions is the feedback between behavior and parasite infestation that may either reinforce or balance the relationship between host and its parasites (Barber & Dingemanse, 2010, Ezenwa, 2016). On the one hand, parasites affect multiple aspects of animal behavior. They cause so-called 'sickness behavior' – an inflammation state induced by cytokines that results in decreased foraging (anorexia), reduced activity and self-grooming (fatigue), diminished aggressiveness, and less interest in social interactions (Kyriazakis et al. 1998, Aubert 1999, Moore 2002). For a long time, such behavior has been considered maladaptive, but it is now recognized as 'behavioral immune system' (Napolioni et al. 2014, Barron et al. 2015), a mechanism by which the host conserves energy, avoids additional pathogens, and combats the infection. Thus, it helps the parasitized animal to decrease parasite loads and return to homeostasis (Moore 2002).

In addition, certain parasites can manipulate host behavior. As a prominent example, animals infested with *Toxoplasma gondii* lose their fear of predators: for instance, Norway rats (*Rattus norvegicus*) with toxoplasmosis cease to avoid cat odor, which benefits the parasite by enhancing its transmission to the definitive host – the feline predator (Berdoy et al. 2000). A similar decrease in predator fear has been observed in house mice (*Mus musculus domesticus*) infected with the nematode *Heligmosomoides polygyrus* and the protozoan *Eimeria verimiformis* (Kavaliers & Colwell, 1995, Kavaliers et al. 1997). Infestation with *H. polygyrus* has also been found to decrease mouse aggression towards conspecifics and to lower their social rank (Barnard et al. 1998). Similarly, three-spined sticklebacks (*Gasterosteus aculeatus*) infected with the microsporidian *Glugea* become more sociable compared to uninfected individuals, potentially facilitating the spread of infection in the population (Petkova et al. 2018).

However, the relationship between host behavior and parasites is reciprocal: animal personality is thought to strongly influence parasite loads and transmission by causing negative or positive feedbacks that eventually affect the co-evolution of host and its parasites (Barber & Dingemanse 2010, Kortet et al. 2010, Ezenwa et al. 2016). That is, hosts that exhibit more locomotory activity, cover longer distances, and occupy larger territories are believed to have a higher chance of gaining more food resources or mates. However, these advantages trade off with a higher risk of encountering free-living parasites or their infective stages (Barber & Dingemanse 2010, Kortet et al. 2010, Ezenwa et al. 2016). For instance, Boyer et al. (2010) found a link between the personality traits of the Siberian chipmunk (*Tamias sibiricus*) and tick burdens: more exploratory individuals carried more ticks than their less mobile counterparts.

Other studies, however, indicate that parasitized animals might reduce their activity to fight infection (Moore 2002). This complexity makes it challenging to separate the cause and effect in the feedback loop between parasite infections and host behaviors, especially when personality and behavioral responses to parasites interact (Barron et al. 2015). However, examining this feedback is crucial for understanding the dynamics of parasite infections and their effects on hosts.

In this study, we aimed to investigate 1) whether individual differences in exploratory behavior, measured in open-field tests, are associated with the infestation of yellow-necked mice (*Apodemus flavicollis*) by two ectoparasite groups: ticks and fleas. Due to significant biological and ecological differences between ticks and fleas, they likely respond differently to host behavior. In addition, we examined 2) whether individuals that display more exploratory behavior in the open-field test differ from their less exploratory counterparts in space use, evaluated with indices derived from live-trapping data. Then, we investigated 3) whether these space use metrics correlate with tick and flea burdens. we also used experimental parasite reduction in the wild to test 4) if parasites modify their hosts' behavior. Specifically, we predicted that:

1) Mice with high exploration scores in the open-field tests would be more prone to tick encounters and infestation, which result in higher tick abundance (defined as the number of parasites on a single host, regardless of whether or not the host is infected: Bush et al. 1997) (**prediction 1a**; see Fig. 1 for an overview of predictions). Castor bean ticks (*Ixodes ricinus*) spend most of their lives off the host. They lay eggs and molt in the environment, then find hosts using cues such as host scent and CO_2 concentration (Wilson et al. 1972, van Duijvendijk et al. 2017). They exhibit questing behavior and ambush their hosts from the vegetation.

   Conversely, we expected fleas to be less abundant on highly exploratory host individuals (**prediction 1b**). Both ecological groups of fleas, nest fleas and fur fleas, rely heavily on host burrows for larvae development (Krasnov 2008). If the exploratory animals cover bigger distances and have larger home ranges, they likely use a greater number of scattered burrows and shelters, visiting each burrow less often. As a consequence, fleas may feed less frequently or have limited access to nests where larvae could safely develop. We expect this to have a negative impact on their abundance on such hosts.

2) Trapping-derived measures, including trappability (a potential indicator of boldness, Boyer et al. 2010, Patterson & Schulte-Hostedde, 2011, Montiglio et al. 2012, Le Coeur et al. 2015, Santicchia et al. 2021) and mean distance covered between captures (an index of mobility found to be associated with home range size, Faust, 1971, Koeppl 1977, Begg et al. 1981, Püttker et al. 2012) would positively correlate with exploration scores from open-field tests conducted on the same mice (**predictions 2a and 2b**, respectively). The theory of animal personality assumes that behavioral traits such as exploration, boldness, and activity correlate creating 'behavioral syndromes', therefore we can expect animals we test in the open field will also behave analogically in the wild (Sih et al. 2004, Spiegel et al. 2017). On the other hand, we did not expect a relationship between trap overlap and open field exploration because the overlap is thought to reflect sociability and contact rates that may result in increased parasite transmission (Robert et al. 2012, Leu et al. 2017, Amaya et al. 2021, Shchipanov

& Demidova 2022), but it is believed to be independent of the shy-bold personality axis (Réale et al. 2007, Gartland et al. 2021; **prediction 2c**).

3) Hosts with greater trappability and mobility indices would experience higher tick infestations (**prediction 3a**), while animals with higher trap overlap scores (presumably a sign of greater sociability) would have more fleas (**prediction 3b**).
4) Finally, we expected that experimental reduction of the ectoparasite burden would increase the activity levels in mice, which might have been suppressed due to ‘sickness behavior’. Specifically, long-term exposure to fleas can cause anemia and decrease the overall condition of the host by depleting resources and impeding their efficient acquisition. Thus, we expected that animals relieved from the parasite pressure could recover and compensate for the lost energy by increasing their open field exploration **(prediction 4a**) and all three indices of spatial activity: trappability (**prediction 4b**), mean distance between captures (**prediction 4c**), and trap overlap (**prediction 4d**).

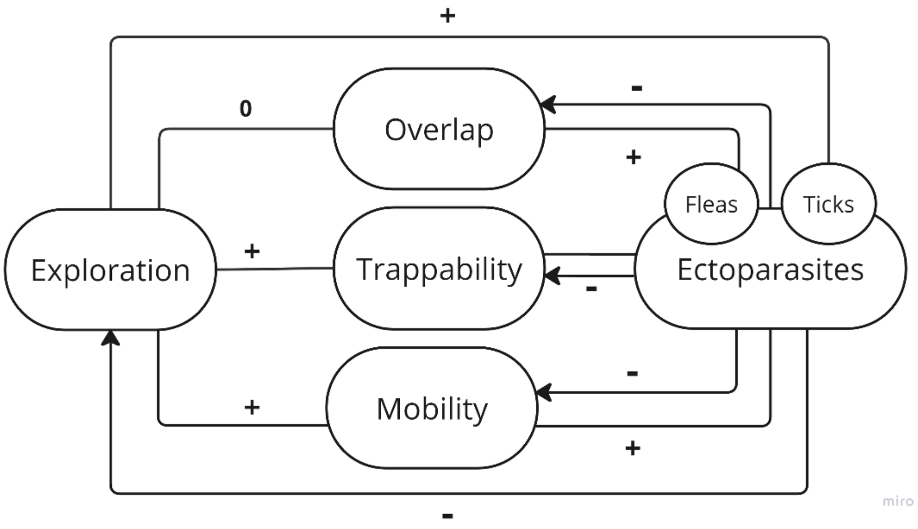

Figure 1. Diagram presenting an overview of the predictions tested in the study. The arrowheads indicate expected causality from experimental manipulation of parasite abundance. Plus and minus indicate hypothesized positive and negative relationships, and ‘0’ indicates no anticipated link. “Exploration’ refers to the rate of open field exploration by yellow-necked mice (*Apodemus flavicollis*). Terms “overlap’, “trappability’, and “mobility’ are behavioral indices derived from trapping data.

METHODS

STUDY SITES

We conducted the research in Forest Inspectorate Łopuchówko, Buczyna District, Greater Poland Voivodeship in N-W Poland. The area is part of the largest forest complex near Poznań, Puszcza Zielonka. The maximum altitude of the study area is 143 m a.s.l. and the landscape is mainly flat or hilly. The climate is temperate, with temperatures ranging from an average of -2.5ºC in January to 18.2ºC in July, and annual precipitation averaging 520 mm. The research was conducted in managed mature forests dominated by European beech (*Fagus sylvatica*), along with other species, such as pedunculate oak *(Quercus robur*), red oak (*Quercus rubra*), European hornbeam (*Carpinus betulus*), sycamore maple *(Acer pseudoplatanus*) and Scots pine *(Pinus sylvestris)*.

SMALL-MAMMAL LIVE-TRAPPING

We established six trapping sites, each containing 100 live traps, arranged in a 10×10 grid with 10-meter spacing between trapping stations (on 8100 m^2 quadrats). The trapping sites were located at least 300 meters apart to minimize mouse movement between them. We conducted trapping over three summer seasons, from the beginning of June to early September, with three (2018-19) or five (2020) trapping sessions each year. Each trapping session lasted five (2018 and 2019) or four (2020) consecutive days, followed by approximately two-week break. In total, we conducted 30,000 trap nights over the three-year study period (9,000 in 2018 and 2019, and 12,000 in 2020). Trapping was held in the morning and in the evening, but behavioral tests, parasite collection and antiparasitic treatment were held only during the morning sessions. As our focal species was *Apodemus flavicollis*, other rodent species were only ear-tagged and weighted. The Eurasian common shrew (*Sorex araneus*) and the Eurasian pygmy shrew (*S. minutus*) were released unmarked. We marked all captured animals with aluminum ear tags with unique ID numbers (National Band and Tag Company, mouse tags type 1005-1) and recorded the species, body mass, sex, and reproductive status of each individual. From 2019, individuals were also provided with peridermal PIT tags for additional identification.

BEHAVIORAL TESTS

To minimize the handling stress, potentially interfering with behavioral tests, we conducted these tests at the beginning of the entire process handling. This occurred just after removing the mouse from the trap, and before marking it. Upon the mouse's first capture, it was directed immediately to the behavioral test. If it was recaptured, and at least two days had passed since the last testing, the individual was tested again. Any other recaptured animals were released after recording only their ID, body mass, and reproductive status.

The tested animal was released into an open field arena, constructed from a 28 × 40 × 34-cm plastic box. The floor was divided into four sections by two low (4.5 cm) perpendicular partitions (see, e.g., Bergeron et al 2013, Montiglio et al 2014, Bednarz & Zwolak 2022). The individual's movement within the arena was recorded for 120 seconds with a handheld digital camera while observers remained still and silent. We used the number of times the individual crossed the plastic partitions between the sectors (referred to as 'crossings') as a proxy of exploration. After each test, the arena was thoroughly cleaned with 70 % alcohol and dried with single-use paper towels. Cotton bags were used only once to prevent the scent of conspecifics from altering test results and to avoid pathogen transmission. The bags were washed at a high temperature without detergent between trappings.

ECTOPARASITE SURVEYS

After conducting the behavioral tests and taking the primary measurements, we thoroughly inspected the host to count the ticks attached to its body. Special attention was given to the head (including the ears and face), paws, and tail. From the ticks found, a random subset of 20 was collected for species identification and preserved in 70 % alcohol for later examination. Furthermore, all fleas found on the host, as well as those in the handling bag, were collected. This method serves as an accurate proxy for determining flea abundance in the yellow-necked mouse (Balaž et al. 2022).

To identify the parasite taxa, we extracted the total genomic DNA from each tick and flea individually using the ammonium hydroxide method (Rijpkema & Bruinink, 1996). The species were then identified by analyzing sequence data from the cytochrome c oxidase subunit I (COI) gene fragment. The sequencing and species identification was conducted by the Molecular Biology Techniques Laboratory at Adam Mickiewicz University in Poznań. They used the Ion Torrent S5 System (Thermo Fisher, USA) to sequence the material and compared the results with GenBank reference sequences for species identification.

ANTIPARASITIC TREATMENT

In 2020, experimental ectoparasite reduction was carried out alongside the standard field procedures. The trapping, parasite sampling, and behavioral protocols remained consistent with those used in previous years. From the trapping sites, we randomly selected three and assigned them to experimental treatment, while the remaining three were assigned to control treatment. On the experimental grids, we applied a treatment using a wide-spectrum topical drug. The dosage was 5 µg of fipronil per 1g of body mass and 6 µg of S-methoprene/1g of body mass (FRONTLINE COMBO® Spot on, fipronil – 100 mg/mL, S-methoprene – 120mg/mL, Merial). This treatment was administered as the final procedure before releasing the individual. Using automatic pipettes, we dispensed the substance onto the scruff of the neck and then rubbed it into the fur. On the control grids, a similar treatment was conducted, but paraffin oil was applied instead of the medication. Paraffin oil is neutral for health and commonly used in pharmacology (Rawlings & Lombard, 2012).

INDICES OF SPACE USE

Space use indices were derived from live-trapping data. Individual trappability was measured by dividing the number of times each mouse was captured within a single live-trapping session by the total trapping occassions (i.e., nights of trapping) for that session. Mean distance covered between captures (an index of individual locomotory activity), was calculated as the total distance traveled by each mouse between consecutive trappings within one session, divided by the number of captures in a session. This involved using trapping locations as coordinates, sorting them by date, and using the ‘as.ltraj’ function from the R package *adehabitatLT* (Calenge, 2006). This index could only be calculated for mice captured at least twice in a session. Trap location overlap, used as an index of sociability, was calculated as the number of other *A. flavicollis* individuals that entered the same trap locations as the focal individual within one trapping session.

Figure 2. Yellow-necked mouse (*Apodemus flavicollis*) in the experimental arena during the open-field test. The number of times animal crossed the plastic partition within 120 seconds was used as a proxy of exploration in statistical analyses.

Figure 3. Yellow-necked mouse (*Apodemus flavicollis*) parasitized by the larvae of sheep tick (*Ixodes ricinus*). Ectoparasite counts of both ticks and fleas were conducted after the open field test.

ETHICAL NOTE

All animal handling procedures were conducted in accordance with the guidelines set by the Polish Society for Laboratory Animal Science (PolLASA). Furthermore, the Local Ethical Committee in Poznań approved all experimental procedures, including parasite and behavioral surveys, as well as the pharmacological treatment of the animals (Approval No. 24/2018).

STATISTICAL ANALYSES

We conducted all analyses in R (R Core Team, 2018, Rstudio Team, 2020) by fitting Generalized Linear Mixed Models (GLMMs, Zuur & Ieno, 2009) in the package *glmmTMB* (Brooks et al. 2017). Model diagnostics, which included visual inspection of QQ-plots and the correlation between residual vs. fitted values, were performed with the *performance* (Lüdecke et al. 2021) and *DHARMa* (Hartig and Hartig, 2017) packages. We also used these packages to test for zero-inflation, overdispersion, temporal autocorrelation, and collinearity among the variables included in the models.

To determine if open field exploration qualifies as a personality trait, we calculated the repeatability of the open-field test scores using GLMMs fitted with the *rptR* package (Stoffel et al. 2017) with a negative binomial distribution. We then calculated the Best Linear Unbiased Predictors (BLUPs) to obtain a single measure that could be later used in models as a response variable. We fitted the number of crossings in the arena as the response variable, with body mass and test number serving as covariates. Experimental site and mouse ID were included as random factors. For each individual, we extracted the random effects from the model and used the intercept values as an index of open field exploration in subsequent analyses.

To address **aim 1** – testing the influence of open field exploration on the tick or flea loads – we used the abundance of each parasite group as the response variable. To ensure that manual parasite removal did not confound the estimates of tick and flea abundance, we only used the initial parasite count from the first capture of each individual. For the tick data, after excluding one outlier (an individual with 290 ticks), we applied a zero-inflated Poisson distribution. For the flea data, we used a negative binomial distribution. Both models included trapping site and mouse ID as random effects. In addition to the exploration rate, explanatory variables included the host's body mass, which has been demonstrated to affect ectoparasite loads in yellow-necked mice (Balaž et al. 2023; Chapter 1 of this dissertation), as well as day and year variables to account for seasonal and annual fluctuations in parasite populations. In this and the following analyses, body mass, space uses indices and mouse abundance indices were centered and scaled using z-scores.

To tackle **aim 2**, which assesses the relationship between space use and open field exploration, we fitted three separate models with trappability (an index of boldness), mean distance travelled in session (an index of mobility) and overlap (indicative of sociability) as response variables. Trappability was modeled using a Gamma distribution with a logit link. Mobility was square root-transformed and modeled with a Gaussian distribution. Overlap was modeled with a zero-inflated negative-binomial distribution. Preliminary data analysis revealed potential non-linear effects of the exploration rate, so we included a quadratic exploration term as a response variable. we retained this term in the models if it improved model fit by ΔAIC > 2 (Burnham & Anderson 2002). we also included mouse body mass and sex because these variables are known to affect rodent spacing behaviors (Ostfeld, 1990, Borowski, 2003, Stradiotto et al. 2009, Wauters et al. 2021). Space use can be season-dependent, therefore we included day (as a numerical variable, reflecting changes over the trapping season: from June to September) and year (as a three-level factor: 2018, 2019, and 2020) to account for these differences. In the overlap model, we also added the number of individual mice trapped per session as an index of population density. We included trapping site and mouse ID as random effects to align with our study design. After diagnostic tests revealed temporal autocorrelation across all three models, we incorporated an AR(1) autocorrelation term.

To investigate the impact of spatial behaviors of yellow-necked mice on their parasite loads (**aim 3**), we ran two models. The first, using a zero-inflated negative binomial distribution, estimated the abundance of ticks, while the second used a negative binomial distribution for flea abundance. The fixed effects contained three spatial measures: trappability, mobility and overlap, as well as host's body mass. The random effects included mouse ID and trapping site.

To address **aim 4** regarding the impact of parasites on open field exploration and space use in mice, we used data collected during the experimental parasite reduction conducted in 2020. First, we assessed the effectiveness of parasite reduction. To do this, we created a dummy treatment variable where all mice trapped on the control sites were assigned a value of 0, while those at treatment sites were given a value of 1, but only after they received their first dose of medicine (they were given a value of 0 before the medication). The treatment was then renewed once per trapping session. we ran separate models for ticks and fleas, fitting their abundances as response variables with a zero-inflated negative-binomial distribution for ticks and a negative binomial distribution for fleas. In addition to the treatment covariate, we included host body mass and day variables. Since the experimental treatment was only carried out in 2020, the year variable was omitted. Random effects included the trapping site and mouse ID.

Then, we examined potential post-treatment changes in mouse behavior by constructing four analogical models with open field exploration (BLUPs of individual mice) and space use (trappability, mobility, and overlap) as response variables and treatment as the explanatory variable. The distributions used were Gaussian, logit-transformed Gaussian, square root-transformed Gaussian, and negative binomial, respectively. All models included body mass as a fixed variable. Additionally, in the case of open field exploration we included a quadratic term of body mass, as Bednarz & Zwolak (2022) found that both the lightest and heaviest yellow-necked mice had the lowest open field exploration scores. Finally, in the overlap model, we controlled for mouse population density by including an index of mouse abundance (the number of individual mice captured at a given trapping grid in a given session). Individual ID and trapping site were included as random effects, with the exception of the exploration model because individual-level BLUPS made mouse ID redundant. We found signs of temporal autocorrelation in the exploration, trappability and mobility models, therefore, an autocorrelation term (AR1) was added to these models.

RESULTS

Parasites: Over the three-year study, we examined 1,169 individual mice for ticks and 1,228 for fleas. Of all the inspected animals, 92% carried ticks, and the average tick abundance was 11.3 ± 15.2 ticks (Mean ± SD). The flea prevalence was 47%, and the average flea abundance was 1.3 ± 2.2. Both parasite groups exhibited pronounced seasonal and annual variations in abundance (Figure 4 and Figure 5).

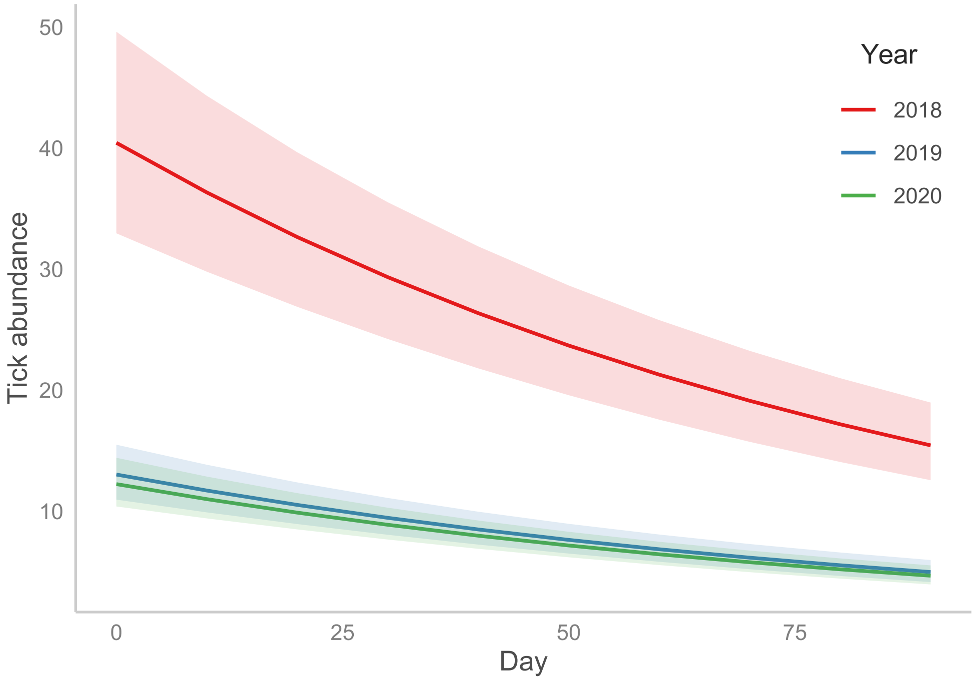

Figure 4. Seasonal and yearly changes in tick infestation on the yellow-necked mouse (*Apodemus flavicollis*) as estimated from a zero-inflated Poisson GLMM (Table 1). The shading corresponds to the 95% confidence interval. Tick abundance was significantly higher in 2018 (red line) compared to 2019 (blue line) and 2020 (green line). Variable ‘day’ represents the changes in tick abundance throughout each trapping season, from June to early September.

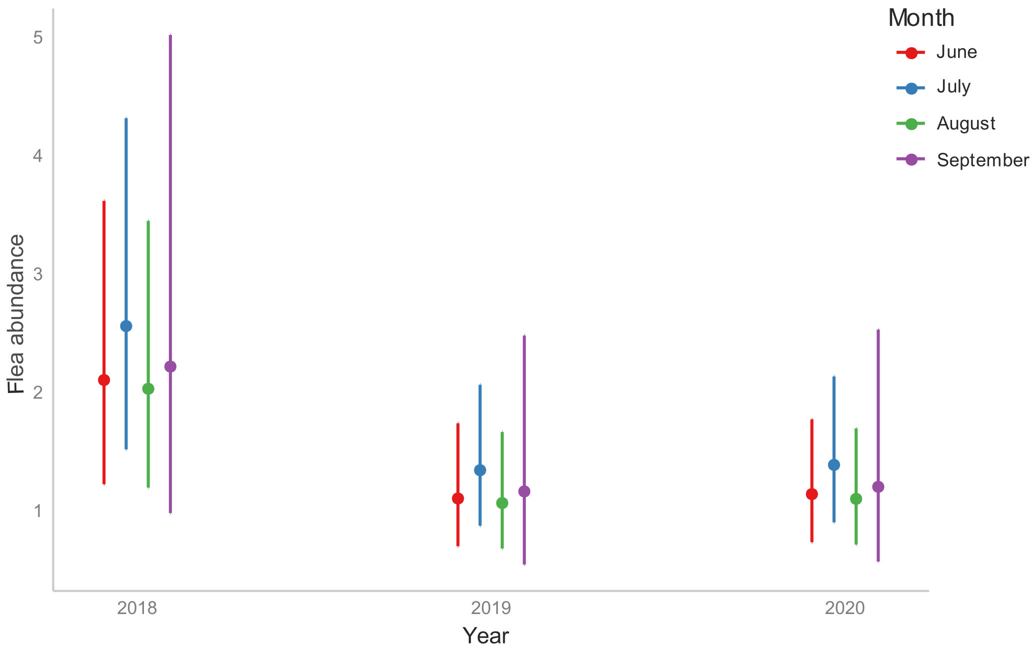

Figure 5. Seasonal and annual variations in flea abundance in yellow-necked mice (*Apodemus flavicollis*) as estimated from a negative binomial, zero-inflated GLMM (Table 2). The dots with bars represent the model estimates along with their 95% CI.

Open field exploration: Throughout the study, we carried out 2,177 behavioral tests on 1,303 individuals, with an average of 1.7 ± 1.1 (Mean ± SD) tests per mouse (519 individuals were tested twice or more). On average, mice crossed the experimental arena 13.4 ± 10.1 times during one open-field test. The adjusted repeatability of exploration, represented by the number of crossings, was 0.57 on the link-scale (95% CI: 0.52–0.62, $p < 0.001$) and 0.46 on the original scale (95% CI: 0.43–0.53, $p < 0.001$).

Space use: In the focal yellow necked-mouse population, the average trappability (defined as the probability of capture per trapping night) was 0.56 ± 0.25 (Mean ± SD). On average, mice moved 16.20 ± 10.27 m between the subsequent captures. The spatial overlap ranged from 0 to 5, with mice sharing traps with an average of 1.17 ± 0.98 other individuals.

1. ASSOCIATION BETWEEN THE OPEN FIELD EXPLORATION AND PARASITE LOADS

TICKS

Tick abundance was positively associated with host's exploration in the open field (Table 1 and Figure 6), supporting **prediction 1a**. According to model estimates, an increase in exploration of one standard deviation above the average was affiliated with hosting 18.5% more ticks. We also found a significant positive effect of host's body mass (Table 1). This association had similar strength: per each standard deviation in mass, the tick abundance increased by 16.2%. Tick loads gradually decreased over time (variable 'day' in Table 1) and varied across years: ticks were significantly more abundant in 2018 compared to the following years (Table 1).

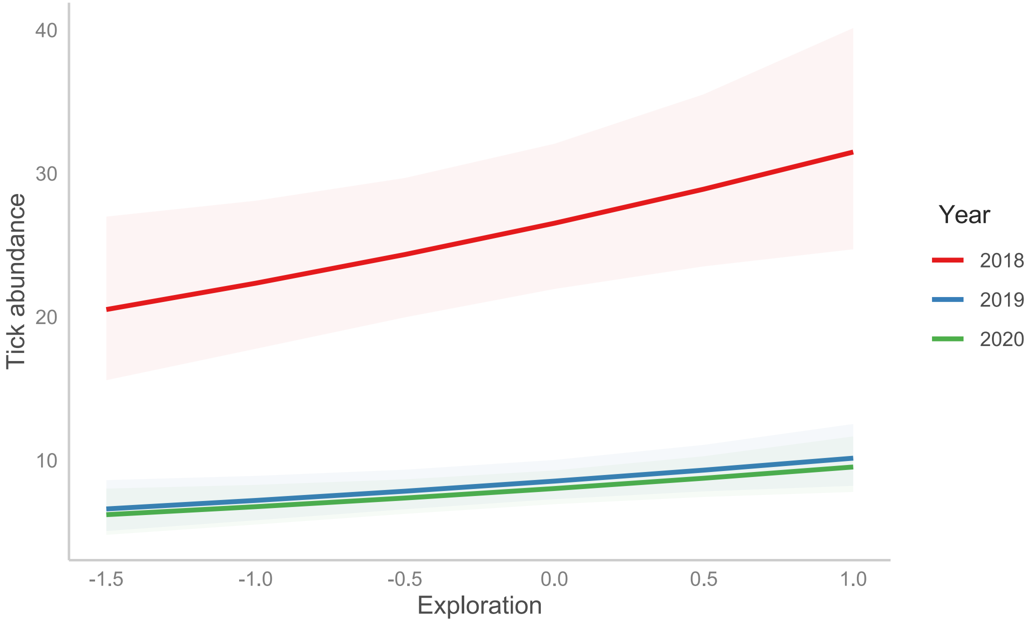

Figure 6. Tick abundance in relation to open field exploration scores (zero-centered and z-score scaled) in yellow-necked mice (*Apodemus flavicollis*) over a three-year study period. Lines with shading correspond to etimates from zero-inflated Poisson GLMM (details in Table 1) and 95% CI.

Table 1. Summary of the GLMM model output testing the association between open field exploration and average tick infestation in yellow-necked mice (*Apodemus flavicollis*). Additional variables included host body mass (z-score scaled), day of the study (reflecting changes in tick abundance over the trapping season), and year (2018 as a reference level).

Variable	**Beta**	**SE**[1]	**95% CI**[1]	**p-value**[2]
(Intercept)	3.694	0.104	3.489, 3.898	**<0.001*** **
Exploration rate	0.172	0.072	0.031, 0.312	**0.016***
Body mass	0.154	0.021	0.112, 0.195	**<0.001*** **
Day	-0.011	0.001	-0.012, -0.009	**<0.001*** **
Year 2019	-1.132	0.078	-1.286, -0.979	**<0.001*** **
Year 2020	-1.195	0.072	-1.337, -1.053	**<0.001*** **

[1] SE = Standard Error, CI = Confidence Interval

[2] *p<0.05; **p<0.01; ***p<0.001

FLEAS

Flea abundance did not change with host exploration in the open field (Table 2), therefore **prediction 1b** was not supported. Flea abundance varied with year but did not change over the trapping season (Table 2). In addition, flea loads were affected by host body mass (Table 2): per each standard deviation of mass, flea abundance increased by 17.4%.

Table 2. Summary of the GLMM model output testing the association between open field exploration and average flea infestation in yellow-necked mice (*Apodemus flavicollis*). Additional variables included host's body mass (z-score scaled), day of the study (reflecting changes in tick abundance over the trapping season), and year (2018 as a reference level).

Variable	**Beta**	**SE**[1]	**95% CI**[1]	**p-value**[2]
(Intercept)	0.737	0.267	0.215, 1.260	**0.006****
Exploration rate	-0.122	0.195	-0.504, 0.260	0.532
Body mass	0.202	0.050	0.103, 0.300	**<0.001*** **
Day	-0.001	0.002	-0.005, 0.004	0.809
Year 2019	-0.573	0.177	-0.921, -0.225	**0.001****
Year 2020	-0.542	0.166	-0.867, -0.216	**0.001****

[1] SE = Standard Error, CI = Confidence Interval

[2] *p<0.05; **p<0.01; ***p<0.001

2. ASSOCIATION BETWEEN EXPLORATION IN THE OPEN FIELD AND SPACE USE

TRAPPABILITY

Trappability of yellow-necked mice was significantly associated with their open field exploration rate (**prediction 2a**). Nevertheless, contrary to our expectation, the relationship was U-shaped rather than linear: individuals with the highest and the lowest levels of open field exploration were trapped more frequently than those displaying intermediate exploration levels (Table 3 and Figure 7). Neither body mass nor sex influenced trappability. Trappability increased throughout the trapping season,, but did not differe between years (Table 3).

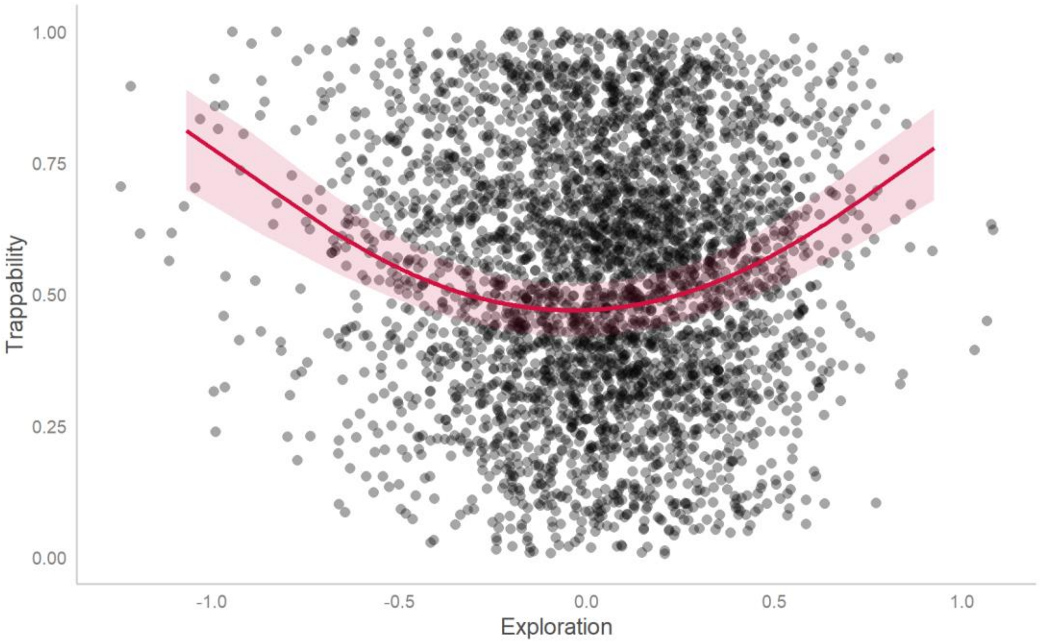

Figure 7. Relationship between exploration in the open field and trappability in yellow-necked mice (*Apodemus flavicollis*). The red line represents GLMM estimates, the shading corresponds to 95% CI, and the black dots represent data points, jittered for better visibility.

Table 3: Summary of the GLMM model output testing the association between trappability and open field exploration in yellow-necked mice (*Apodemus flavicollis*). Additional variables included host's body mass (z-score scaled), day of the study (reflecting changes in tick abundance over the trapping season), and year (2018 as a reference level).

Variable	**Beta**	**SE**[1]	**95% CI**[1]	**p-value**[2]
(Intercept)	-0.242	0.137	-0.511, 0.027	0.078
Exploration rate	0.473	1.91	-3.279, 4.225	0.805
Exploration rate^2	12.336	2.18	8.063, 16.609	**<0.001*** **
Body mass	0.024	0.025	-0.026, 0.073	0.354
Sex (males)	0.000	0.054	-0.106, 0.106	0.995
Day	0.006	0.002	0.002, 0.010	**0.005** **
Year 2019	0.044	0.095	-0.143, 0.231	0.645
Year 2020	-0.100	0.092	-0.281, 0.081	0.277

[1] SE = Standard Error, CI = Confidence Interval

[2] *p<0.05; **p<0.01; ***p<0.001

MOBILITY

The mobility of mice, measured by the mean distance between captures, was not associated with open field exploration (Table 4), contrary to our **prediction 2b**. However, males covered significantly longer distances on the trapping grid compared to females (Table 4). Furthermore, mobility varied with year, but did not change over the trapping season and did not depend on the body mass (Table 4).

Table 4. Summary of the GLMM model output examining the association between the mean distance travelled between captures (mobility) of yellow-necked mice (*Apodemus flavicollis*) and their exploration in the open-field test. Additional variables included host's body mass (z-score scaled), sex (females as reference level), day of the study (reflecting changes over the trapping season), and year (2018 as a reference level).

Variable	**Beta**	**SE**[1]	**95% CI**[1]	**p-value**[2]
(Intercept)	4.225	0.201	3.831, 4.620	**<0.001*** **
Exploration rate	-0.231	0.181	-0.585, 0.124	0.202
Body mass	-0.004	0.038	-0.078, 0.071	0.922
Sex (males)	0.211	0.107	0.001, 0.422	**0.049***
Day	0.000	0.002	-0.005, 0.005	0.918
Year 2019	-0.463	0.169	-0.794, -0.132	**0.006** **
Year 2020	-0.429	0.166	-0.756, -0.103	**0.010** **

[1] SE = Standard Error, CI = Confidence Interval

[2] *p<0.05; **p<0.01; ***p<0.001

SPATIAL OVERLAP

Contrary to **prediction 2c**, the overlap index was positively associated with the open-field exploration (Table 5 and Figure 8): a one standard deviation increase in the exploration score was associated with a 17.4% rise in the overlap index. The overlap was positively correlated with population density and varied across years, but did not change over the trapping season (Table 5). The effects of sex and body mass were non-significant (Table 5).

Table 5. Summary of the GLMM model output examining the association between the overlap of trapping locations with conspecifics ('sociability') of yellow-necked mice (*Apodemus flavicollis*) and their exploration in the open-field test. Additional variables included host's body mass (z-score scaled), sex (females as reference level), day of the study (reflecting changes over the trapping season), and year (2018 as a reference level).

Variable	**Beta**	**SE**[1]	**95% CI**[1]	**p-value**[2]
(Intercept)	-0.581	0.123	-0.822, -0.339	**<0.001***
Exploration rate	0.157	0.058	0.044, 0.270	**0.006**
Body mass	0.017	0.018	-0.018, 0.051	0.340
Sex (males)	0.030	0.033	-0.035, 0.096	0.366
Day	0.001	0.002	-0.002, 0.004	0.468
Year 2019	0.748	0.103	0.546, 0.951	**<0.001***
Year 2020	0.431	0.089	0.256, 0.606	**<0.001***
A.flavicollis abundance	0.096	0.028	0.040, 0.151	**<0.001***

[1] SE = Standard Error, CI = Confidence Interval

[2] *p<0.05; **p<0.01; ***p<0.001

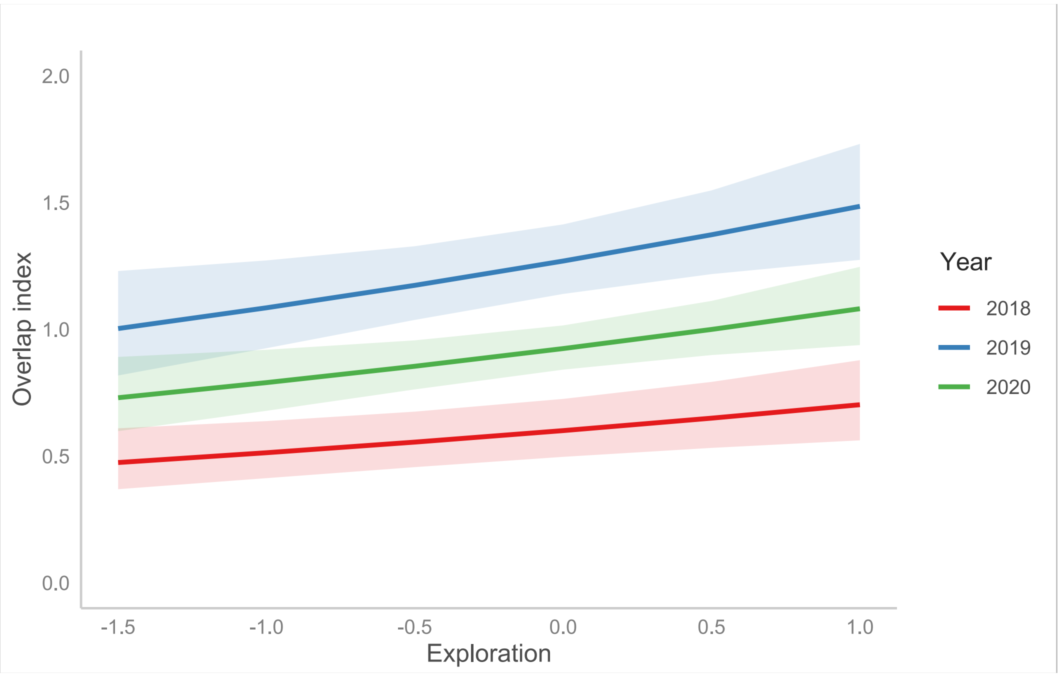

Figure 8. Relationship between open field exploration of yellow-necked mice (*Apodemus flavicollis*) and their sociability, measured as the number of conspecifics that entered the same traps within one trapping session. Lines with shading represent estimates from GLMM with 95% CI over three years of the study: 2018 (red), 2019 (blue) and 2020 (green).

3. ASSOCIATION BETWEEN SPACE USE AND PARASITE LOADS

TICKS

Tick abundance was not associated with the spatial behavior of their host, therefore **prediction 3a** was not supported. There was no relationship of tick loads with trap overlap, nor mobility, while the negative correlation with trappability only approached significance (Table 6). Other variables in the model (body mass, day, and year) followed patterns described in section 1. of the ‘Results’ (Table 6).

Table 6. Summary of the GLMM model output examining the association between the average tick infestation of yellow-necked mice (*Apodemus flavicollis*) and indices of their space use (trappability, mobility, and overlap – all scaled). Additional variables included host's body mass (scaled), day of the study (reflecting changes over the trapping season), and year (2018 as a reference level).

Variable	Beta	SE[1]	95% CI[1]	p-value[2]
(Intercept)	3.859	0.193	3.480, 4.237	**<0.001*****
Trappability	-0.097	0.054	-0.203, 0.008	0.071
Overlap	-0.041	0.038	-0.116, 0.033	0.278
Mobility	-0.053	0.056	-0.162, 0.056	0.339
Body mass	0.110	0.052	0.008, 0.211	**0.034***
Day	-0.009	0.002	-0.014, -0.004	**<0.001*****
Year 2019	-1.212	0.150	-1.506, -0.919	**<0.001*****
Year 2020	-1.285	0.152	-1.584, -0.987	**<0.001*****

[1] SE = Standard Error, CI = Confidence Interval

[2] *p<0.05; **p<0.01; ***p<0.001

FLEAS

Flea loads showed no correlation with any of the spatial measures we considered, contradicting prediction 3b. Neither overlap, trappability, nor mobility impacted flea abundance (Table 7). Other covariates followed patterns similar to those described in section 1. of the 'Results' (details in Table 7).

Table 7: Summary of the GLMM model output examining the association between the average flea infestation of yellow-necked mice (Apodemus flavicollis) and indices of their space use (trappability, mobility, and overlap). Additional variables included host's body mass (scaled), day of the study (reflecting changes over the trapping season), and year (2018 as a reference level).

Variable	Beta	SE[1]	95% CI[1]	p-value[2]
(Intercept)	0.436	0.300	-0.151, 1.023	0.146
Trappability	-0.082	0.092	-0.264, 0.099	0.373
Overlap	0.070	0.106	-0.137, 0.277	0.508
Mobility	0.104	0.088	-0.068, 0.276	0.235
Body mass	0.165	0.092	-0.016, 0.345	0.074
Day	-0.005	0.004	-0.013, 0.003	0.264
Year 2019	-0.497	0.263	-1.011, 0.018	0.059
Year 2020	-0.541	0.255	-1.041, -0.042	**0.034***

[1] SE = Standard Error, CI = Confidence Interval

[2] *p<0.05; **p<0.01; ***p<0.001

4. IMPACT OF PARASITE LOADS ON BEHAVIOR OF THE HOST

The antiparasitic treatment reduced the abundance of both ticks and fleas in yellow-necked mice, with the effects intensifying over time (Figure 9). For each day that passed, the tick abundance on treated mice was reduced by an additional 2.2%, and flea abundance by 2.3% compared to the control group (Treatment × Day interaction in Table 8). By the end of the experiment (day 83), this led to estimated reductions of 82.6% for ticks and 89.6% for fleas.

Table 8. Summary of the GLMM model output examining the effect of antiparasitic treatment on the average tick and flea infestation of yellow-necked mice (*Apodemus flavicollis*). The 'Treatment' variable denotes two-level factor with animals before treatment and after receiving placebo as 'Control' (as reference level in the model) and animals that received FRONTLINE COMBO® Spot on as 'Treatment'. Additional variables included host's body mass (scaled) and day of the study (reflecting changes over the trapping season).

	Ticks			*Fleas*		
Variable	**Beta**	**95% CI[1]**	**P**	**Beta**	**95% CI[1]**	**P**
(Intercept)	2.486	2.286, 2.686	**<0.001**	-0.011	-0.708, 0.686	0.975
Treatment (Frontline)	0.080	-0.317, 0.475	0.696	-0.353	-1.494, 0.788	0.544
Body mass	0.201	0.158, 0.245	**<0.001**	0.256	0.149, 0.364	**<0.001**
Day	-0.008	-0.010, -0.006	**<0.001**	-0.001	-0.006, 0.004	0.750
Treatment * Day	-0.022	-0.030, -0.015	**<0.001**	-0.023	-0.044, -0.002	**0.028**

[1] CI = Confidence Interval

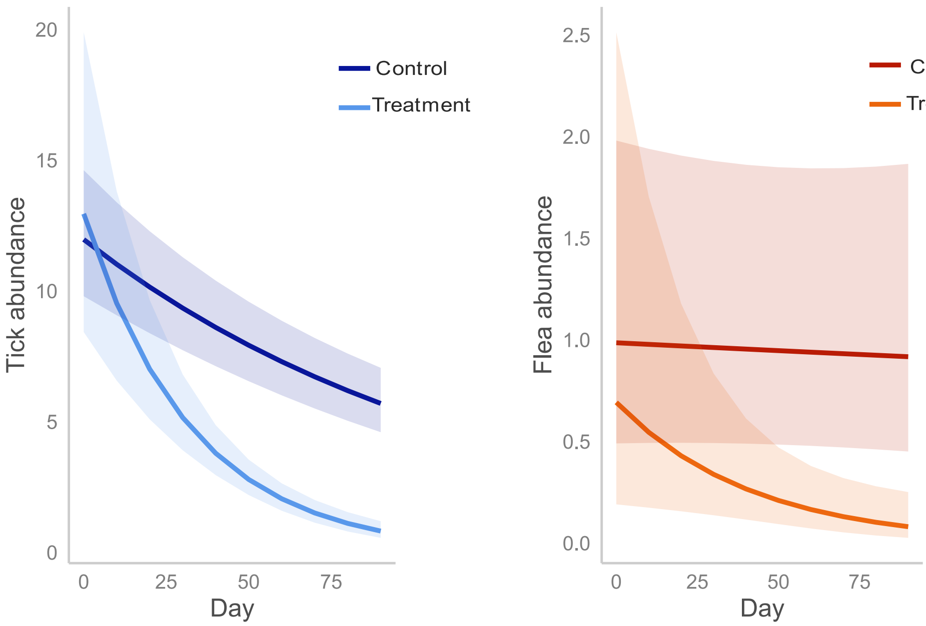

Figure 9. Effects of antiparasitic treatment on the abundance of ticks (blue) and fleas (red) of yellow-necked mice (*Apodemus flavicollis*). Darker lines represent parasite numbers in mice that were treated with placebo (or untreated, in the case of first captures), while the lighter ones indicate individuals treated with FRONTLINE COMBO® Spot on. The shading corresponds to 95% CI. The estimates are derived from two GLMMs that use either tick or flea average abundance as response variables (see Table 6 for summaries).

OPEN FIELD EXPLORATION

After the antiparasitic treatment, the exploration rates of yellow-necked mice did not differ from those of individuals who received the placebo treatment (Table 9), thus **prediction 4a** was not supported. Open field exploration scores were non-linearly associated with body mass: the smallest and largest mice had lower exploration scores compared to average-sized individuals (Table 9). Moreover, there was a significant increase in the exploration rates across the trapping season (Table 9).

Table 9. Summary of the GLMM model output examining the effect of antiparasitic treatment the on open field exploration of yellow-necked mice (*Apodemus flavicollis*). Additional variables included host's body mass (quadratic, scaled) and day of the study (reflecting changes over the trapping season).

Variable	**Beta**	**SE**[1]	**95% CI**[1]	**p-value**[2]
(Intercept)	0.079	0.044	-0.007, 0.164	0.072
Treatment	0.033	0.039	-0.043, 0.109	0.393
Body mass	-1.216	0.314	-1.830, -0.601	**<0.001*** **
Body mass²	-1.782	0.316	-2.401, -1.164	**<0.001*** **
Sex (males)	-0.011	0.013	-0.037, 0.015	0.401
Day	0.001	0.0004	-0.001, 0.000	0.081
Treatment*Day	-0.001	0.001	-0.002, 0.001	0.238

[1] SE = Standard Error, CI = Confidence Interval

[2] *p<0.05; **p<0.01; ***p<0.001

TRAPPABILITY

Trappability significantly increased among animals that received the antiparasitic medicine compared to those administered placebo, (Table 10 and Figure 10), providing support for **prediction 4b**. In addition, trappability grew over time, but was unaffected by both mouse sex and body mass (Table 10).

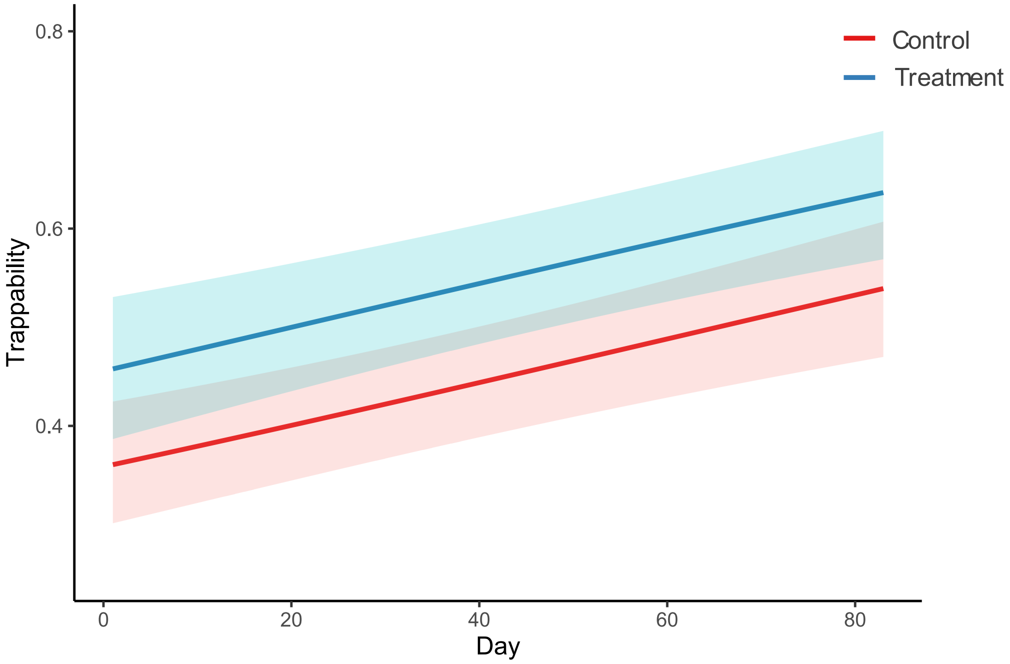

Figure 10. Effects of the antiparasitic treatment on the trappability of the yellow-necked mouse (*Apodemus flavicollis*). The lines represent GLMM estimates, and the shading corresponds to 95% CI (see Table 9 for summary).

Table 10. Summary of the GLMM model output examining the effect of antiparasitic treatment on the trappability of yellow-necked mice (*Apodemus flavicollis*) Additional variables included host's body mass (scaled), sex, and day of the study (reflecting changes over the trapping season).

Variable	**Beta**	**SE**[1]	**95% CI**[1]	**p-value**[2]
(Intercept)	-0.599	0.146	-0.884, -0.314	**<0.001*****
Treatment	0.503	0.143	0.223, 0.783	**<0.001*****
Body mass	0.012	0.034	-0.055, 0.078	0.728
Sex (males)	-0.010	0.072	-0.152, 0.131	0.886
Day	0.010	0.002	0.006, 0.013	**<0.001*****
Treatment*Day	-0.002	0.003	-0.008, 0.003	0.409

[1] SE = Standard Error, CI = Confidence Interval

[2] *p<0.05; **p<0.01; ***p<0.001

MOBILITY

The average distance moved between the traps (mobility) was not influenced by the antiparasitic treatment, therefore **prediction 4c** was not supported (Table 11). Furthermore, none of the other tested variables had an impact on the mobility index (Table 11).

Table 11. Summary of the GLMM model output examining the effect of antiparasitic treatment on the mean distance moved between captures of yellow-necked mice (*Apodemus flavicollis*) Additional variables included host's body mass (scaled), sex, and day of the study (reflecting changes over the trapping season).

Variable	**Beta**	**SE**[1]	**95% CI**[1]	**p-value**[2]
(Intercept)	4.227	0.204	3.827, 4.627	**<0.001*****
Treatment	-0.010	0.163	-0.328, 0.309	0.953
Body mass	0.079	0.068	-0.054, 0.212	0.245
Sex (males)	-0.093	0.169	-0.424, 0.237	0.580
Day	0.001	0.002	-0.004, 0.006	0.643
Treatment*Day	-0.002	0.003	-0.008, 0.004	0.570

[1] SE = Standard Error, CI = Confidence Interval

[2] *p<0.05; **p<0.01; ***p<0.001

SPATIAL OVERLAP

The overlap in trapping locations (an index of sociability) remained unchanged after the antiparasitic treatment (Table 12), thus **prediction 4d** was not supported. Population density was the most important determinant of the overlap (Table 12 and Figure 11). Neither body mass, sex nor season influenced the sociability of yellow-necked mice (Table 12).

Table 12. Summary of the GLMM model output examining the effect of antiparasitic treatment on the overlap of trapping locations for yellow-necked mice (*Apodemus flavicollis*) Additional variables included the host's body mass (scaled), sex, day of the study (reflecting changes over the trapping season), and the number of captured yellow-necked mice as a proxy of population density.

Variable	**Beta**	**SE**[1]	**95% CI**[1]	**p-value**[2]
(Intercept)	-0.018	0.140	-0.292, 0.255	0.895
Treatment	-0.071	0.214	-0.490, 0.347	0.738
Body mass	0.018	0.040	-0.061, 0.097	0.658
Sex (males)	0.010	0.080	-0.146, 0.165	0.904
Day	0.000	0.002	-0.004, 0.004	0.914
Treatment*Day	0.002	0.003	-0.004, 0.009	0.486
A.flavicollis abundance	0.095	0.036	0.024, 0.166	**0.009****

[1] SE = Standard Error, CI = Confidence Interval

[2] *p<0.05; **p<0.01; ***p<0.001

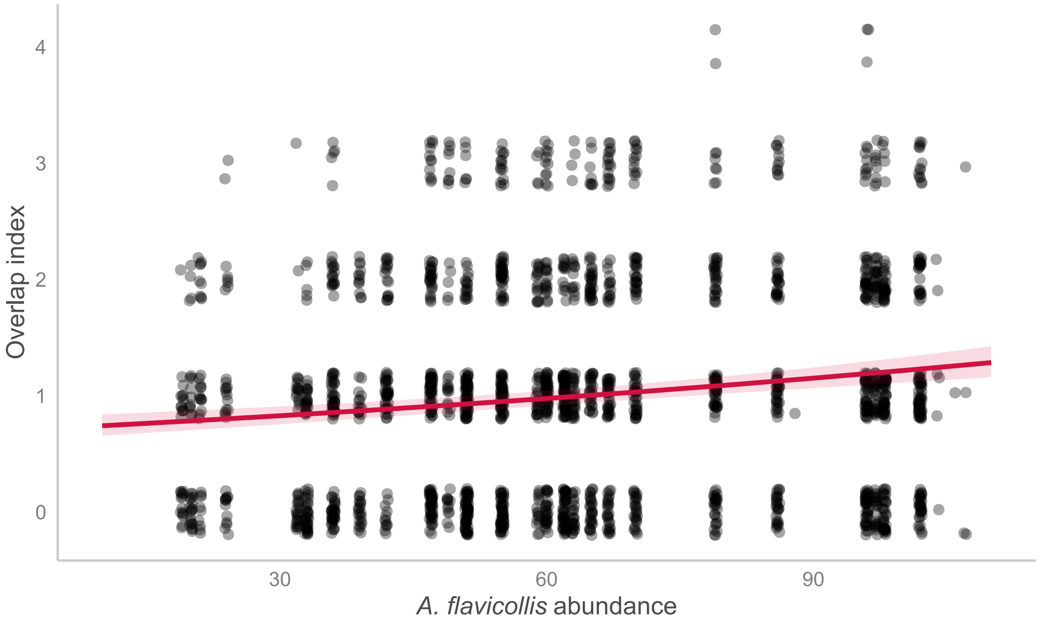

Figure 11. Effects of yellow-necked mouse abundance (measured as the number of individuals trapped within one session at a focal trapping grid) on the trapping location overlap (an index of sociability). Red line with shading represents estimates from GLMM with 95% CI, and black dots represent data points, jittered for better visibility. See Table 11 for model summary.

DISCUSSION

The study provided partial support for the predictions we made. The relationship between ectoparasite abundance and open field exploration or space use varied depending on the parasite taxon. we observed an association between the behavior of yellow-necked mice and their tick infestations (significant for open field exploration and approaching significance for trappability); however, no such association was observed with flea infestations.

Moreover, experimental parasite reduction revealed that ectoparasite infestation affects mouse trappability. Untreated mice had a lower capture probability compared to their treated conspecifics, supporting the notion of reduced activity pattern known as 'sickness behavior' and suggesting the existence of negative feedback loops between host behavior and its ectoparasites. Yet, other behavioral aspects we assessed, including open field exploration, sociability, and activity, remained unchanged after the parasite reduction. Thus, the connections between host behavior and parasite loads hinge on the specific behaviors and parasite groups in consideration (see Table 13 and Figure 12 for summary).

1. HOST PERSONALITY AND PARASITE BURDENS

Our findings indicate that fleas and ticks respond differently to their host's personality, which is likely due to their distinct biological and ecological attributes. Specifically, only tick abundance was associated with host open field exploration scores (supporting **prediction 1a** but not **1b**: Table 13). This mirrors findings from Boyer et al. (2010), who found a positive correlation between exploration scores of Siberian chipmunks (*Tamias striatus)* in hole-board tests, and their tick loads; similar results were documented by Bohn et al. 2017 for least chipmunks (*T. minimus*). On the other hand, other studies suggest that this relationship between host personality and tick parasitism is not universally consistent. For example, studies on rock elephant shrews *(Elephantulus myurus*) (Hoffmann, 2014), Barbery ground squirrels (*Atlantoxerus getulus*) (Piquet et al. 2018), and sleepy lizards (*Tiliqua rugosa*) (Sih et al. 2018, Payne et al. 2019, 2020, 2022) found varied or no correlations.

Table 13. Summary of tested predictions and results.'OFT' refers to the open-field test.

		Prediction	Supported?	Result
Effect of personality on parasites	**1.**	a. Higher OFT exploration → higher tick abundance	Yes	Positive association between exploration in the open-field test and tick abundance
		b. Lower OFT exploration → higher flea abundance	No	No relationship between exploration in the open-field test and flea abundance
Effect of personality on space use	**2.**	a. Higher OFT exploration → higher trappability	Partly	U-shaped relationship between open field exploration and trappability; intermediate exploration levels were associated with the lowest trappability
		b. Higher OFT exploration → higher mobility	No	No relationship between open field exploration and mobility in the trapping grid
		c. Higher OFT exploration → lower sociability (overlap)	No	Positive correlation between the trap overlap and open field exploration
Space use effect on parasites	**3.**	a. Higher spatial activity (trappability, mobility) → more ticks	No	The association between trappability and tick abundance was negative, but marginally non-significant. No link between mobility and tick abundance.
		b. Higher sociability (overlap) → more fleas	No	No relationship between spatial overlap and flea abundance
Parasite effect on personality and behavior	**4.**	a. Treatment → OFT exploration increased	No	No effect of the antiparasitic treatment on OFT exploration
		b. Treatment → increased trappability	Yes	Trappability increased after the antiparasitic treatment
		c. Treatment → increase mobility	No	The antiparasitic treatment had no effect on mobility
		d. Treatment → increased sociability (overlap)	No	The antiparasitic treatment had no effect on the spatial overlap

The data on fleas did not align with **prediction 1b** (Table 13). Flea abundance seems to be unrelated to the personality of the yellow-necked mouse. A potential reason could be that lower exploration scores in mice do not necessarily translate to longer time spent in their nest, which might otherwise boost flea abundance. Moreover, behavioral tendencies might be overshadowed by other host traits such as sex or reproductive status (Garrido et al. 2016, Aliperti et al. 2021, Wauters et al. 2021). Thus, it could be beneficial for future research to account for the complex interplay of personality across various sexes and reproductive phases. However, in this study, we chose not to pursue this angle in order to maintain clarity in predictions and avoid building overly complex models.

There is a scarcity of literature linking flea burdens to host personality, and the results are ambiguous. For instance, a study on the least chipmunk suggested a positive association between ectoparasite abundance and host exploration in hole-board tests (Bohn et al. 2017). However, since this study pooled results for several ectoparasite groups (ticks, fleas, and mites), it is impossible to draw specific conclusions about flea responses. Another investigation by Caron-Lévesque & Careau (2023), revealed differences in how ticks and fleas infesting *Peromyscus* mice are associated with the host personality. Specifically, fleas were more abundant in animals exhibiting more active behavior in open-field tests, but this was only evident in the absence of ticks. Interactions between ectoparasites were beyond the scope of this study, but it is conceivable that factors like competition, facilitation, or host-mediated interactions can modify host-parasite relationships (Krasnov et al. 2010, Karbowiak et al. 2019). Such complexities merit in-depth investigation in upcoming studies.

2. PERSONALITY AND SPACE USE

Our study provided new insights into open field exploration in yellow-necked mice, offering a glimpse into how this behavior translates – or not – into space use. Exploration was linked with trappability (**prediction 2a**), but in a non-linear pattern: both the most and least exploratory animals had the highest probability of capture. It is possible that there is an intrinsic state-dependent inclination for risky foraging behavior. The most exploratory individuals may be able to “afford’ to take risky decisions, as a consequence of boldness and efficiency in acquiring resources. The least active individuals, on the other hand, may be forced to take risky decisions because of low energy intake. Moreover, it is possible that ‘shy’ individuals are pushed to lower quality habitat patches (Schirmer et al. 2019) and must compensate by feeding in more risky locations, such as traps.

Mobility, estimated in our study as the average distance between consecutive captures, did not show any association with open field exploration (**prediction 2b**). This indicates that these two measures probably reflect different aspects of behavior.

Finally, more exploratory animals showed greater territorial overlap (contrary to prediction 2c). Exploration has often been found to be associated with boldness and aggression, but its relationship with sociability varies from study to study (Sih et al. 2004; Réale et al. 2010; Gartland et al. 2021, and references therein). Even though personality research has undergone many methodological improvements over the last decade, such mixed results may be due to the fact that defining which personality axis is measured (boldness, exploration, activity, sociality) in a given behavioral test is still debated, and the interpretations of behaviors exhibited by different species may vary substantially. In the case of our focal species, *A. flavicollis*, individuals that scored higher in the open-field test also entered traps visited by their conspecifics more often than the less exploratory individuals. However, the more traps an individual visits, the more likely it is, simply by chance, that such individuals will cross paths. Therefore, it could be beneficial to correct the overlap index by accounting for the number of trapping locations (trap diversity) in future analyses as well as apply the mirror stimulation test - standard aggression-sociality measure (Svendsen & Armitage 1973).

In past studies, there were several attempts to bridge animal behavior in standardized tests such as the open field arena with indices derived from trapping data. These also brought varying results. Jolly et al. (2019) did not find any links between open field exploration and trappability measures in the grassland melomys (*Melomys burtoni*). Similarly, there was no link between exploration in behavioral tests and trappability in the multimammate mouse (*Mastomys natalensis*) (Vanden Broecke et al. 2021), but it is worth noting that these studies might have overlooked a non-linear relationships. In contrast, Boyer et al. (2010) discovered that exploration conducted in the hole-board test is reflected by trappability and trap diversity measures (and these, in turn, affect tick loads).

In another study, Aliperti et al. (2022) identified a positive relationship between boldness (assessed via flight initiation distance) and the size of core areas in golden-mantled ground squirrels (*Callospermophilus lateralis*). Interestingly, individuals that were more active in the open-field test were also more sociable (as assessed by the mirror image stimulation test), in agreement with our findings. In addition, a study on two *Peromyscus* species. showed significant among-individual correlation between struggle and exploration times, but none of the behavioral measures (exploration in the open field, struggle time, and latency to emerge from the trap) were associated with home range size (Underhill et al. 2021). Finally, a study by Brehm & Mortelliti (2018) showed that indices derived from trapping data, such as latency to first capture, trap diversity and overall trappability, are not repeatable and therefore cannot be used as a proxy for personality.

In conclusion, even though space use cannot be used as a direct proxy for personality, it offers valuable insights on the mechanisms by which open-field tests relate to behaviors in natural environment. The challenge lies in interpreting the diverse outcomes across studies to identify overarching trends.

3. HOST SPACE USE AND PARASITE BURDENS

Contrary to expectations, there was no significant correlation between the three indices of spatial activity and ectoparasite loads of yellow-necked mice. The mobility (the average distance travelled between consecutive captures) was not correlated with ectoparasite abundance, which was unexpected, as we anticipated this measure to reflect an exploratory tendency in the wild (**prediction 3a** in Table 13). However, this rather coarse metric may underestimate the variation in movement between individuals and therefore be inadequate to detect more subtle differences. Moreover, the trappability showed a negative trend that was approaching significance (**prediction 3a** assumed a positive association). There was also no discernible correlation between the overlap and ectoparasite abundance (contrary to **prediction 3b**).

How do these findings compare to other research? Boyer et al. (2010) found a positive effect of trappability and trap diversity on tick loads in Siberian chipmunks. In our research, trap diversity (the number of trap locations visited by the individual in a session) was not analyzed because it was highly correlated with trappability, therefore it would not serve as an independent index of space use. Conversely, in the white-footed mouse (*Peromyscus leucopus*), the relationship between the tick load and movement rate was negative (Gaitan & Millen, 2016). The authors stated that decreased mobility leads to a lower chance for highly parasitized mice to expand or shift their home ranges, and that such less mobile individuals face lower risk of tick parasitism when foraging within their own home range.

In contrast to the equivocal results on the relationship between spatial behaviors and ectoparasites, research on intestinal endoparasites often shows more consistent patterns. A study on eastern grey squirrels (*Sciurus carolinensis*) found that ‘bolder’ (more trappable) individuals have higher prevalence of endoparasites than their ‘shyer’ (less trappable) conspecifics. However, the intensity of infestation was only affected by the host body mass (Santicchia et al. 2019). Among wood mice (*Apodemus sylvaticus*), male mice that have larger home ranges and spend higher proportion of time on the move were more frequently parasitized by the nematode *Heligmosomoides polygyrus* (Brown et al. 1994). Still, since gastrointestinal worms maintain a much stronger relationship with their hosts compared to ectoparasites – most of them being highly specialized, obligatory endoparasites – it is difficult to generalize the mechanisms that shape the populations of these different parasite groups.

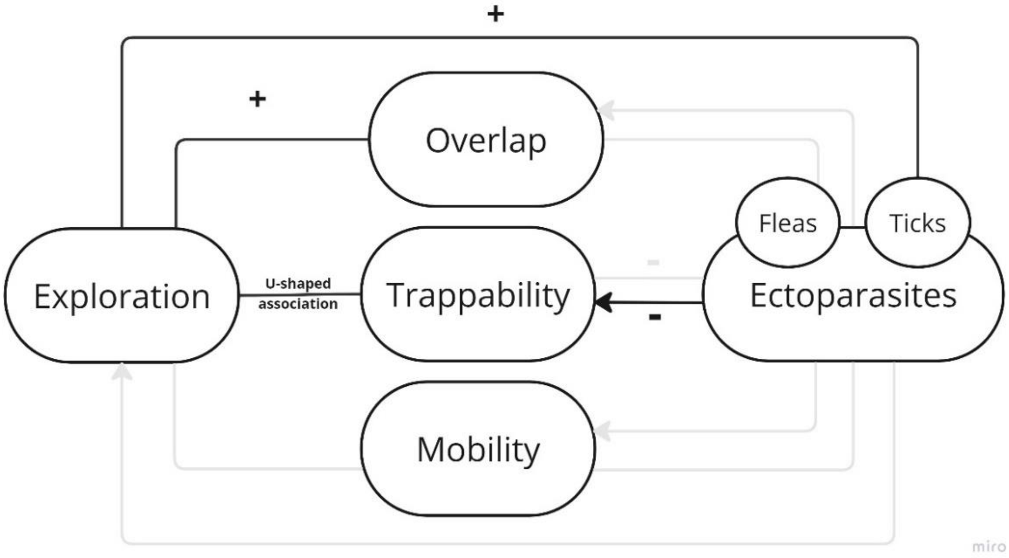

Figure 12. Diagram presenting an overview of the associations tested in the study. Supported links are marked with black, and unsupported – with grey lines. The arrowheads indicate causality demonstrated in the experimental manipulation of parasite abundance. Plus and minus indicate positive and negative relationships, respectively. “Exploration’ refers to the rate at which yellow-necked mice (*Apodemus flavicollis*) explore in the open-field tests. Terms “overlap’, “trappability’, and “mobility’ are behavioral indices derived from trapping data.

4. FEEDBACKS BETWEEN HOST BEHAVIOR AND PARASITE BURDENS

According to Hawley & Ezenwa (2022), two conditions must be met to define bidirectional interaction between host behavior and its parasites as a functional feedback loop: (1) animal behavior must affect parasitism parameters, such as encounter probability, reproduction, transmission, and (2) the parasites must respond to that change and reinforce it. For instance, Tasmanian devils (*Sarcophilus harrisii*) get infected with Devil Facial Tumor Disease (DFTD) via social contacts with other individuals. However, once infected, they tend to lower the frequency of social interactions, which in turn reduces the further infection risk and helps to bring the system back to homeostasis (Hamilton et al. 2020).

We observed analogical patterns in our study for trappability: the trappability of yellow-necked mice tended to decrease when heavily infested by the ticks and increased when their parasite burden was reduced. Possibly, more parasitized individuals decrease their risky activities to conserve energy and protect themselves from further infestation. In turn, higher trappability, which is often interpreted as a proxy of boldness and risk-taking, may expose mice to more parasites again. Therefore, with our experimental approach we were able to capture the balancing nature of the feedback between mouse trappability and tick abundance. Yet, none of the other behavioral metrics evaluated in this study formed a bidirectional link with parasite abundance. Even though sociability (trap overlap) was linked with exploration in the open-field tests, it had no effect on parasite loads and did not respond to the antiparasitic treatment. Similarly, the influence of mobility was non-significant in all models we fitted.

As a caveat, we cannot conclusively determine which ectoparasite groups directly impacted trappability after the treatment because antiparasitic drugs affect a wide spectrum of ectoparasites. Nevertheless, observational data indicate that tick, and not flea abundance, are negatively associated with trappability.

CONCLUSIONS

Over recent decades, the burgeoning interest in the ecological importance of animal personalities has spurred numerous studies. These have showcased intriguing links between standardized behavioral metrics obtained in controlled settings and various ecological variables in the wild, including parasite burdens. Unlike many studies that rely on cross-sectional observational data, our research combined observational and experimental approaches to probe the relationship between host behavior and parasite abundance.

We hypothesized that a specific personality trait – exploration in the open field – would influence host space use, which would then influence parasite loads. However, this mechanism was partly confirmed only for ticks, not for fleas. We found that the abundance of ticks on yellow-necked mice changed with host trappability, but not mobility or trap overlap. Additionally, the experimental manipulation of parasite loads affected trappability, and not other behavioral indices. If we interpret trappability as an indicator of risk-taking behavior (see e.g., Boyer et al. 2010, Patterson & Schulte-Hostedde, 2011, Montiglio et al. 2012, Le Coeur et al. 2015, Santicchia et al. 2021), it suggests that this trait might be pivotal in shaping the relationship between the mice and the ticks. This behavior could also be instrumental in reinforcing the negative feedback within the system, a crucial stabilizing force in host-parasite interactions.

REFERENCES

Aliperti, J. R., Davis, B. E., Fangue, N. A., Todgham, A. E., & Van Vuren, D. H. (2021). Bridging animal personality with space use and resource use in a free-ranging population of an asocial ground squirrel. *Animal Behaviour*, *180*, 291-306.

Amaya, J. P., Cuello, P. A., Valentinuzzi, V. S., & Lacey, E. A. (2021). Dynamic spatial overlap in a solitary subterranean rodent: the Anillaco tuco-tuco (*Ctenomys sp*.). *Journal of Mammalogy*, *102*(3), 826-836.

Aubert, A. (1999). Sickness and behaviour in animals: a motivational perspective. *Neuroscience & Biobehavioral Reviews*, *23*(7), 1029-1036.

Barber, I., & Dingemanse, N. J. (2010). Parasitism and the evolutionary ecology of animal personality. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1560), 4077-4088.

Barnard, C. J., Behnke, J. M., Gage, A. R., Brown, H., & Smithurst, P. R. (1998). The role of parasite–induced immunodepression, rank and social environment in the modulation of behaviour and hormone concentration in male laboratory mice (*Mus musculus*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *265*(1397), 693-701.

Barron, D. G., Gervasi, S. S., Pruitt, J. N., & Martin, L. B. (2015). Behavioral competence: how host behaviors can interact to influence parasite transmission risk. *Current Opinion in Behavioral Sciences*, *6*, 35-40.

Bednarz, P. A., & Zwolak, R. (2022). Body mass and sex, but not breeding condition and season, influence open-field exploration in the yellow-necked mouse. *Ecology and Evolution*, *12*(3), e8771.

Begg, R. J. (1981). The small mammals of Little Nourlangie Rock, NT III. Ecology of *Dasyurus hallucatus*, the northern quoll (Marsupialia: Dasyuridae). *Wildlife Research*, *8*(1), 73-85.

Berdoy, M., Webster, J. P., & Macdonald, D. W. (2000). Fatal attraction in rats infected with *Toxoplasma gondii*. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *267*(1452), 1591-1594.

Bergeron, P., Montiglio, P. O., Réale, D., Humphries, M. M., Gimenez, O., & Garant, D. (2013). Disruptive viability selection on adult exploratory behaviour in eastern chipmunks. *Journal of Evolutionary Biology*, *26*(4), 766-774.

Bohn, S. J., Webber, Q. M., Florko, K. R., Paslawski, K. R., Peterson, A. M., Piche, J. E., ... & Willis, C. K. (2017). Personality predicts ectoparasite abundance in an asocial sciurid. *Ethology*, *123*(10), 761-771.

Borowski, Z. (2003). Habitat selection and home range size of field voles *Microtus agrestis* in Słowiński National Park, Poland. *Acta Theriologica*, *48*, 325-333.

Boyer, N., Réale, D., Marmet, J., Pisanu, B., & Chapuis, J. L. (2010). Personality, space use and tick load in an introduced population of Siberian chipmunks *Tamias sibiricus*. *Journal of Animal Ecology*, *79*(3), 538-547.

Brehm, A. M., & Mortelliti, A. (2018). Mind the trap: large-scale field experiment shows that trappability is not a proxy for personality. *Animal Behaviour*, *142*, 101-112.

Brooks, M. E., Kristensen, K., Van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., ... & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R journal*, *9*(2), 378-400.

Brown, E. D., Macdonald, D. W., Tewand, T. E., & Todd, I. A. (1994). *Apodemus sylvaticus* infected with *Heligmosomoides polygyrus* (Nematoda) in an arable ecosystem: epidemiology and effects of infection on the movements of male mice. *Journal of Zoology*, *234*(4), 623-640.

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *The Journal of Parasitology*, 575-583.

Butler, J. M., & Roper, T. J. (1996). Ectoparasites and sett use in European badgers. *Animal Behaviour*, *52*(3), 621-629.

Calenge, C. (2006) The package adehabitat for the R software: a tool for the analysis of space and habitat use by animals. Ecological Modelling, 197, 516-519

Dawkins, R., & Krebs, J. R. (1979). Arms races between and within species. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, *205*(1161), 489-511.

Diamond, J. (1986). Laboratory experiments, field experiments, and natural experiments. *Community Ecology*, 3-22.

Faust, B. F., Smith, M. H., & Wray, W. B. (1971). Distances moved by small mammals as an apparent function of grid size. *Acta Theriologica*, *16*(11), 161-177.

Ezenwa, V. O., Archie, E. A., Craft, M. E., Hawley, D. M., Martin, L. B., Moore, J., & White, L. (2016). Host behaviour–parasite feedback: an essential link between animal behaviour and disease ecology. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1828), 20153078.

Gaitan, J., & Millien, V. (2016). Stress level, parasite load, and movement pattern in a small-mammal reservoir host for Lyme disease. *Canadian Journal of Zoology*, *94*(8), 565-573.

Garrido, M., Adler, V. H., Pnini, M., Abramsky, Z., Krasnov, B. R., Gutman, R., ... & Hawlena, H. (2016). Time budget, oxygen consumption and body mass responses to parasites in juvenile and adult wild rodents. *Parasites & Vectors*, 9(1), 1-14.

Gooderham, K., & Schulte-Hostedde, A. (2011). Macroparasitism influences reproductive success in red squirrels (*Tamiasciurus hudsonicus*). *Behavioral Ecology*, *22*(6), 1195-1200.

Habig, B., & Archie, E. A. (2015). Social status, immune response and parasitism in males: a meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1669), 20140109.

Hamilton, D. G., Jones, M. E., Cameron, E. Z., Kerlin, D. H., McCallum, H., Storfer, A., ... & Hamede, R. K. (2020). Infectious disease and sickness behaviour: tumour progression affects interaction patterns and social network structure in wild Tasmanian devils. *Proceedings of the Royal Society B*, *287*(1940), 20202454.

Harrison, A., Scantlebury, M., & Montgomery, W. I. (2010). Body mass and sex-biased parasitism in wood mice *Apodemus sylvaticus*. *Oikos*, *119*(7), 1099-1104.

Hartig, F., & Hartig, M. F. (2017). Package 'DHARMa'. *R package*.

Hawley, D. M., & Ezenwa, V. O. (2022). Parasites, host behavior, and their feedbacks. *Animal Behavior and Parasitism*, 15.

Hoffmann, S. (2014). *Personality, hormonal correlates and parasite load in the eastern rock elephant shrew* (Doctoral dissertation, University of Pretoria).

Jolly, C. J., Webb, J. K., Gillespie, G. R., Hughes, N. K., & Phillips, B. L. (2019). Bias averted: personality may not influence trappability. *Behavioral Ecology and Sociobiology*, *73*, 1-11.

Karbowiak, G., Miklisová, D., Stanko, M., Werszko, J., Hajdul-Marwicz, M., Szewczyk, T., & Rychlik, L. (2019). The competition between immatures of *Ixodes ricinus* and *Dermacentor reticulatus* (Ixodida: Ixodidae) ticks for rodent hosts. *Journal of medical entomology*, *56*(2), 448-452.

Kavaliers, M., & Colwell, D. D. (1995). Discrimination by female mice between the odours of parasitized and non-parasitized males. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *261*(1360), 31-35.

Kavaliers, M., Colwell, D. D., Ossenkopp, K. P., & Perrot-Sinal, T. S. (1997). Altered responses to female odors in parasitized male mice: neuromodulatory mechanisms and relations to female choice. *Behavioral Ecology and Sociobiology*, *40*, 373-384.

Koeppl, J. W., Slade, N. A., & Hoffmann, R. S. (1977). Distance between observations as an index of average home range size. *American Midland Naturalist*, 476-482.

Kortet, R., Hedrick, A. V., & Vainikka, A. (2010). Parasitism, predation and the evolution of animal personalities. *Ecology Letters*, *13*(12), 1449-1458.

Krasnov, B. R. (2008). *Functional and Evolutionary Ecology of Fleas: a model for ecological parasitology*. Cambridge University Press.

Krasnov, B. R., Matthee, S., Lareschi, M., Korallo-Vinarskaya, N. P., & Vinarski, M. V. (2010). Co-occurrence of ectoparasites on rodent hosts: null model analyses of data from three continents. *Oikos*, *119*(1), 120-128.

Kyriazakis, I., Tolkamp, B. J., & Hutchings, M. R. (1998). Towards a functional explanation for the occurrence of anorexia during parasitic infections. *Animal Behaviour*, *56*(2), 265-274.

Le Coeur, C., Thibault, M., Pisanu, B., Thibault, S., Chapuis, J. L., & Baudry, E. (2015). Temporally fluctuating selection on a personality trait in a wild rodent population. *Behavioral Ecology*, *26*(5), 1285-1291.

Loker, E. S., & Hofkin, B. V. (2022). *Parasitology: a conceptual approach*. CRC Press.

Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P., & Makowski, D. (2021). performance: An R package for assessment, comparison and testing of statistical models. *Journal of Open Source Software*, *6*(60).

Montiglio, P. O., Garant, D., Pelletier, F., & Réale, D. (2012). Personality differences are related to long-term stress reactivity in a population of wild eastern chipmunks, *Tamias striatus*. *Animal Behaviour*, *84*(4), 1071-1079.

Montiglio, P. O., Garant, D., Bergeron, P., Messier, G. D., & Réale, D. (2014). Pulsed resources and the coupling between life-history strategies and exploration patterns in eastern chipmunks (*Tamias striatus*). *Journal of Animal Ecology*, *83*(3), 720-728.

Moore, J. (2002). *Parasites and the Behavior of Animals*. Oxford University Press.

Napolioni, V., Murray, D. R., Comings, D. E., Peters, W. R., Gade-Andavolu, R., & MacMurray, J. (2014). Interaction between infectious diseases and personality traits: ACP1* C as a potential mediator. *Infection, Genetics and Evolution*, *26*, 267-273.

Neuhaus, P. (2003). Parasite removal and its impact on litter size and body condition in Columbian ground squirrels (*Spermophilus columbianus*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(suppl_2), S213-S215.

Ostfeld, R. S. (1990). The ecology of territoriality in small mammals. *Trends in Ecology & Evolution*, *5*(12), 411-415.

Patterson, L. D., & Schulte-Hostedde, A. I. (2011). Behavioural correlates of parasitism and reproductive success in male eastern chipmunks, Tamias striatus. *Animal Behaviour*, *81*(6), 1129-1137.

Payne, E. M. (2019). *Integrating Consistent Among-Individual Differences in Behavior and Parasite Load in a Wild Population of Sleepy Lizards, Tiliqua rugosa*. University of California, Davis.

Payne, E., Sinn, D. L., Spiegel, O., Leu, S. T., Wohlfeil, C., Godfrey, S. S., ... & Sih, A. (2020). Consistent individual differences in ecto-parasitism of a long-lived lizard host. *Oikos*, *129*(7), 1061-1071.

Payne, E., Spiegel, O., Sinn, D. L., Leu, S. T., Gardner, M. G., Godfrey, S. S., ... & Sih, A. (2022). Intrinsic traits, social context, and local environment shape home range size and fidelity of sleepy lizards. *Ecological Monographs*, *92*(3), e1519.

Petkova, I., Abbey-Lee, R. N., & Løvlie, H. (2018). Parasite infection and host personality: *Glugea*-infected three-spined sticklebacks are more social. *Behavioral Ecology and Sociobiology*, *72*, 1-9.

Piquet, J. C., López-Darias, M., van der Marel, A., Nogales, M., & Waterman, J. (2018). Unraveling behavioral and pace-of-life syndromes in a reduced parasite and predation pressure context: personality and survival of the Barbary ground squirrel. *Behavioral Ecology and Sociobiology*, *72*, 1-12.

Poirotte, C., Massol, F., Herbert, A., Willaume, E., Bomo, P. M., Kappeler, P. M., & Charpentier, M. J. (2017). Mandrills use olfaction to socially avoid parasitized conspecifics. *Science Advances*, *3*(4), e1601721.

Püttker, T., De Barros, C. D. S., Martins, T. K., Sommer, S., & Pardini, R. (2012). Suitability of distance metrics as indexes of home-range size in tropical rodent species. *Journal of Mammalogy*, *93*(1), 115-123.

Rawlings, A. V., & Lombard, K. J. (2012). A review on the extensive skin benefits of mineral oil. *International Journal of Cosmetic Science*, *34*(6), 511-518.

Reckardt, K., & Kerth, G. (2007). Roost selection and roost switching of female Bechstein's bats (*Myotis bechsteinii*) as a strategy of parasite avoidance. *Oecologia*, *154*, 581-588.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews*, *82*(2), 291-318.

Rijpkema, S., & Bruinink, H. (1996). Detection of *Borrelia burgdorferi* sensu lato by PCR in questing *Ixodes ricinus* larvae from the Dutch North Sea island of Ameland. *Experimental & Applied Acarology*, *20*, 381-385.

Robert, K., Garant, D., & Pelletier, F. (2012). Keep in touch: does spatial overlap correlate with contact rate frequency?. *The Journal of Wildlife Management*, *76*(8), 1670-1675.

Santicchia, F., Romeo, C., Ferrari, N., Matthysen, E., Vanlauwe, L., Wauters, L. A., & Martinoli, A. (2019). The price of being bold? Relationship between personality and endoparasitic infection in a tree squirrel. *Mammalian Biology*, *97*, 1-8.

Shchipanov, N. A., & Demidova, T. B. (2022). Inter-annual fluctuations of sociability in the common shrew (*Sorex araneus* L.) as determined by a preference test: A case of balancing selection?. *Behavioural Processes*, *198*, 104625.

Sih, A., Spiegel, O., Godfrey, S., Leu, S., & Bull, C. M. (2018). Integrating social networks, animal personalities, movement ecology and parasites: a framework with examples from a lizard. *Animal Behaviour*, *136*, 195-205.

Spiegel, O., Leu, S. T., Bull, C. M., & Sih, A. (2017). What's your move? Movement as a link between personality and spatial dynamics in animal populations. *Ecology Letters*, *20*(1), 3-18.

Stoffel, M. A., Nakagawa, S., & Schielzeth, H. (2017). rptR: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *8*(11), 1639-1644.

Stradiotto, A., Cagnacci, F., Delahay, R., Tioli, S., Nieder, L., & Rizzoli, A. (2009). Spatial organization of the yellow-necked mouse: effects of density and resource availability. *Journal of Mammalogy*, *90*(3), 704-714.

Svendsen, G. E., & Armitage, K. B. (1973). Mirror-image stimulation applied to field behavioral studies. *Ecology*, *54*(3), 623-627.

Team, R. C. (2018). A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. *Available online: www. R-project. org*

Team, R. (2020). RStudio: Integrated Development Environment for R (RStudio, PBC, Boston, MA, 2020). *Available online: www. R-project. org*

Tompkins, D. M., & Begon, M. (1999). Parasites can regulate wildlife populations. *Parasitology Today*, *15*(8), 311-313.

Underhill, V., Pandelis, G. G., Papuga, J., Sabol, A. C., Rife, A., Rubi, T., ... & Dantzer, B. (2021). Personality and behavioral syndromes in two *Peromyscus* species: presence, lack of state dependence, and lack of association with home range size. *Behavioral Ecology and Sociobiology*, *75*, 1-16.

Vanden Broecke, B., Sluydts, V., Mariën, J., Sabuni, C. A., Massawe, A. W., Matthysen, E., & Leirs, H. (2021). The effects of personality on survival and trappability in a wild mouse during a population cycle. *Oecologia*, *195*, 901-913.

van Duijvendijk, G., Gort, G., Sprong, H., & Takken, W. (2017). Behavioural responses of *Ixodes ricinus* nymphs to carbon dioxide and rodent odour. *Medical and Veterinary Entomology*, *31*(2), 220-223.

Wauters, L. A., Mazzamuto, M. V., Santicchia, F., Martinoli, A., Preatoni, D. G., Lurz, P. W., ... & Romeo, C. (2021). Personality traits, sex and food abundance shape space use in an arboreal mammal. *Oecologia*, *196*, 65-76.

Weinstein, S. B., Buck, J. C., & Young, H. S. (2018). A landscape of disgust. *Science*, *359*(6381), 1213-1214.

Wilson, J. G., Kinzer, D. R., Sauer, J. R., & Hair, J. A. (1972). Chemo-attraction in the lone star tick (Acarina: Ixodidae): I. Response of different developmental stages to carbon dioxide administered via traps. *Journal of Medical Entomology*, *9*(3), 245-252.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R* (Vol. 574, p. 574). New York: springer.

FIGURES

TABLES

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I declare that the work in the research article Zduniak M., Serafini S., Wróbel A., Zwolak R. “Host body mass, not sex, affects ectoparasite loads in yellow-necked mouse *Apodemus flavicollis”* doi: https://doi.org/10.1007/s00436-023-07958-5 of which I am a first co-author, is a part of my PhD.

MZ and SS contributed equally. SS, MZ, RZ conceived and designed the study. MZ, SS, AW collected the data. SS, MZ, AW processed and prepared data for analysis. SS, RZ and MZ analyzed the data. SS and MZ wrote the manuscript. SS prepared figure 1 and 2, MZ prepared figure 3 and Supplementary Materials. AW and RZ provided critical feedback and helped shape the manuscript. All authors read and approved the final manuscript.

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I declare that I am aware that the work in the research article Zduniak M., Serafini S., Wróbel A., Zwolak R. “Host’s body mass, not sex, affects ectoparasite loads in yellow-necked mouse *Apodemus flavicollis”* doi: https://doi.org/10.1007/s00436-023-07958-5 of which I am a co -author, is a part of the PhD dissertation by Milena Zduniak.

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for the research article: **Zduniak M., Wróbel A., Celebias P., Zwolak R. "Personality, space use and ectoparasites: risk-taking behavior drives negative feedback loops between yellow-necked mice (*Apodemus flavicollis*) and ticks (*Ixodes ricinus*)",** unpublished

I declare that research article Zduniak M., Wróbel A., Celebias P., Zwolak R. "Personality, space use and ectoparasite loads: risk-taking behavior drives negative feedback loops between yellow-necked mice (*Apodemus flavicollis*) and ticks (*Ixodes ricinus*)" of which I am a first author, is a part of my PhD dissertation.

MZ, RZ conceived and designed the study. MZ, AW, PC collected the data. MZ, AW, PC processed and prepared data for analysis. MZ and RZ analyzed the data. MZ wrote the manuscript. RZ and AW provided critical feedback and helped shape the manuscript. All authors read and approved the final manuscript.

Date: 28.08.2023

Name: Milena Zduniak

Signature: Milena Zduniak

Supervisor: Rafał Zwolak

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I declare that I am aware that the work in the research article Zduniak M., Wróbel A., Celebias P., Zwolak R. “Personality, space use and ectoparasite loads: risk-taking behavior drives negative feedback loops between yellow-necked mice (*Apodemus flavicollis*) and ticks (*Ixodes ricinus*)*”* of which I am a co-author, is a part of the PhD dissertation by Milena Zduniak.

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Date: 6.09.2023

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CO-AUTHORSHIP STATEMENT

for the research article: **Zduniak M., Wróbel A., Celebias P., Zwolak R. "Personality, space use and ectoparasites: risk-taking behavior drives negative feedback loops between yellow-necked mice (*Apodemus flavicollis*) and ticks (*Ixodes ricinus*)"**

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