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Research paper

Parasitemia and body condition in sympatric and allopatric *Podarcis muralis* populations

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Abstract

Parasitemia and body condition in sympatric and allopatric Podarcis muralis *populations.* Sympatrically distributed species provide natural model systems for investigating inter-host differences in parasitic infections. In this study, 40 individuals (20♂♂, 20♀♀) of *Podarcis muralis* from two different populations (sympatric and allopatric) were compared to examine the effects of population type on intraerythrocytic parasite prevalence and intensity, body condition (Scaled Mass Index), and H/L ratios. Statistically significant differences in body condition between males and females were found in both populations through intra-population comparisons. When sympatric and allopatric populations were compared, parasite intensity differed significantly in both sexes. However, a statistically significant difference in body condition was detected only in females. According to the results, parasite intensity was higher in the sympatric population, while sympatric females exhibited higher body condition values compared to allopatric females. Additionally, parasite intensity was positively correlated with body size in males from both populations, and a strong negative correlation between body condition and H/L ratio was observed in sympatric females. Our findings indicate that parasite intensity is associated with population type, whereas body condition is related to sex.

Key words: Intraerythrocytic parasite, Scaled mass index, Common wall lizard, Sympatry, Allopatry

Introduction

In natural populations, parasites are considered strong drivers of natural selection as they influence the general health status of their hosts and alter the structure of ecological communities (Altizer et al 2003, Beldomenico et al 2008, Sagonas et al 2016). At the individual level, parasitic infections can impair development and growth, thereby reducing the host's fitness (Goater and Ward 1992, Poulin 1999, de Jong-Brink et al 2001). At a broader scale, parasites play a crucial role in population persistence by affecting host abundance (Scott and Dobson 1989), reproduction (Engelstädter and Hurst 2009), and survival (Ebert et al 2000, Žagar et al 2022). Sympatric or closely related species provide natural model systems for investigating interspecific differences in parasitic infections (Thomas et al 1995). Since closely related species share common ancestors, sympatric species may also be more susceptible to the same or similar parasite types due to shared ecological factors (Poulin 2011, Maia et al 2014). However, parasites can potentially shape natural selection depending on host physiology and ecological conditions and differentially affect the fitness of sympatric species (Schall 1992). This can influence competitive interactions and serve as a biological marker of stress in competing hosts (Dajčman et al 2021).

Reptiles have become model organisms for parasitological studies due to their low dispersal potential and the sympatric speciation (Camargo et al 2010, Maia et al 2014). When individuals share basking or shelter sites, they frequently encounter ticks and mites, which act as vectors for blood parasites (Telford 2009). Reptiles are frequently infected with hemogregarines, a widespread and most common group of blood parasites belonging to the phylum Apicomplexa (Telford 2009). The genus *Podarcis*, which includes small wall lizards native to Europe and North Africa, is known to host various parasite genus from both the suborders Adeleorina (e.g., Hemogregarina, Hepatozoon, Karyolysus) and Eimeriorina (e.g., Schellackia, Lankesterella) (Maia et al 2014, Faria et al 2024). Owing to their accessibility, ecological breadth, and well-documented susceptibility to blood parasites, *Podarcis* lizards have been widely used in research on host–parasite interactions in natural populations (Amo et al 2005b, Sagonas et al 2016, Lazić et al 2017, Dajčman et al 2021, Storniolo et al 2022, Žagar et al 2022, Megía-Palma et

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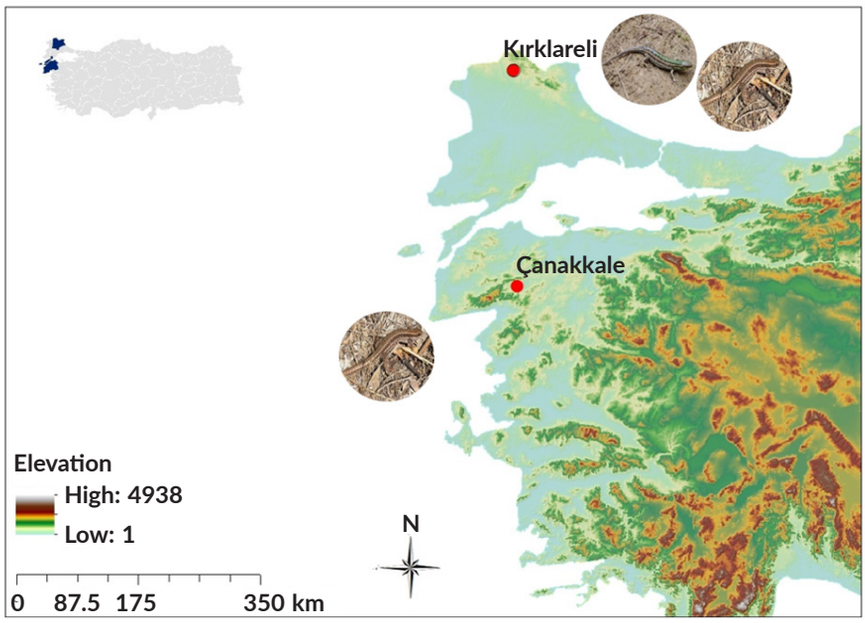

Fig. 1. Studied populations: Kırklareli, sympatric population; Çanakkale, allopatric population.

al 2024). Despite this, blood parasite studies in Türkiye remain scarce: existing research is limited to a few terrapin and snake species (Dvořáková et al 2014, Ceylan et al 2023a, 2023b). To our knowledge, no published work has yet investigated blood parasites in lizards.

In lizards, blood parasites can negatively impact fitness by reducing running speed and slowing tail regeneration (Oppliger and Clobert 1997, Garrido and Pérez-Mellado 2014, Žagar et al 2022). The gametocytes of these parasites damage erythrocytes, thereby decreasing oxygen transport capacity (Oppliger et al 1999). Consequently, this can impair muscle response in activities such as foraging, ultimately leading to a decline in body condition (Wozniak et al 1996, Damas-Moreira et al 2022). Body condition serves as a measure of an organism's energetic and physiological status, reflecting both individual health and population viability (Schulte-Hostedde et al 2005, Peig and Green 2010, Ofori et al 2023). The Scaled Mass Index (SMI) is a reliable and unbiased method for assessing body condition, accounting for allometric changes in body shape during growth (Peig and Green 2009, 2010). This index is significantly correlated with body fat content in ectotherms (MacCracken and Stebbings 2012, Falk et al 2017), and it has been reported that it can serve as a consistent indicator of the effects of environmental stress factors on natural populations, such as parasitic infections (Peig and Green 2009, Maceda-Veiga et al 2014, Megía-Palma et al 2024). Another commonly used method to determine the effects of physiological stress is leukocyte profiles, which are evaluated by the relative proportions of different types of white blood cell (WBC) types (Davis et al 2008, Davis and Maney 2018). Since each leukocyte type plays a distinct role in immune function, variations in their relative abundance are considered indicators of immune responses to stress (Davis et al 2008). In recent years, reptilian immune responses to parasitic infections have been increasingly assessed through leukocyte ratios, particularly heterophil-to-lymphocyte (H/L) ratios (Sperry et al 2009, Davis and Maney 2018).

This study compares 40 individuals (20♂♂, 20♀♀) of *Podarcis muralis* (common wall lizard) from two different populations (sympatric and allopatric) to investigate the effects of population type on intraerythrocytic parasite prevalence and intensity, body condition (SMI), and H/L ratios. We hypothesized that (i) parasite prevalence and intensity would be higher in the sympatric population, (ii) body condition would differ between populations depending on parasite load, and (iii) individuals with higher parasite loads would exhibit elevated H/L ratios. However, if no significant relationship is detected between parasite intensity and body condition, it may reflect a developed tolerance in individuals to intraerythrocytic parasites.

Material and methods

The common wall lizard *Podarcis muralis* (Laurenti 1768) is a small lacertid lizard with distribution across Europe, spanning from the Iberian Peninsula to Türkiye and from Southern Italy and Greece to Northern France (Sillero et al 2014). In Türkiye, it is found in various regions, including the Gallipoli Peninsula, Thrace Region, Northwestern Anatolia, and around Ankara (Baran et al 2021). In the Thrace region, two species of the genus *Podarcis* (*P. muralis* and *P. tauricus*) coexist sympatrically (Baran et al 2021).

Two *P. muralis* populations (sympatric and allopatric) from different stations were studied. In Kırklareli station, North Thrace (41° 55' 0.93" N, 27° 10' 17.06" E, fig. 1), *P. muralis* and *P. tauricus* are abundant and coexisting sympatrically (Baycan et al 2024). In Çanakkale station, southeastern part (39° 48' 47.02'' N, 27° 12' 55.91'' E,

Table 1. Descriptive statistics of the variables employed to describe relationships in the sympatric and allopatric *Podarcis muralis*. * Statistically significant differences between sexes in intrapopulation.

Sympatric population

	10 ♂♂		10 ♀♀	
	Min-Max	Mean ± SE	Min-Max	Mean ± SE
SVL (mm)	47.07-65.65	58.05 ± 1.88	46.44-64.18	57.41 ± 2.08
BW (g)	2.60-6.38	4.63 ± 0.40	1.78-5.62	4.06 ± 0.40
Parasite intensity	0-95	25.50 ± 9.07	0-188	38.70 ± 18.40
SMI*	3.68-5.42	4.54 ± 0.17	3.18-4.99	3.94 ± 0.16
H/L (%)	0.95-5.00	0.86 ± 0.47	0.15-3.50	0.87 ± 0.30

Allopatric population

	10 ♂♂		10 ♀♀	
	Min-Max	Mean ± SE	Min-Max	Mean ± SE
SVL (mm)	53.17-61.31	57.45 ± 0.84	53.29-59.63	55.88 ± 0.67
BW (g)*	3.37-5.96	4.57 ± 0.23	3.18-4.49	3.51 ± 0.13
Parasite intensity	0-19	5.70 ± 2.05	0-16	4.70 ± 1.72
SMI*	3.63-5.16	4.55 ± 0.15	3.06-3.69	3.50 ± 0.06
H/L (%)*	0.14-0.79	0.36 ± 0.06	0.36-2.5	1.04 ± 0.20

fig. 1), there was an allopatric population of *P. muralis*, with no other sympatrically coexisting lizard populations in this region. In both study sites, lizards were generally found on rocky substrates, including large rock blocks, boulder piles, and talus in forested areas. Field studies were conducted from March to mid-April of 2023, before the peak breeding season. A total of 40 sexually mature lizards (snout-vent length >45 mm, Martín-Vallejo et al 1995) were collected, including 20 individuals (10♂♂, 10♀♀) from Kırklareli and 20 (10♂♂, 10♀♀) from Çanakkale.

Blood samples were collected from post orbital sinuses of lizards using a 2 mL syringe with a 21-gauge needle (Thrall et al 2004). Wright-stained blood smears were prepared from the blood samples and examined using an Olympus CX21 microscope. To determine intraerythrocytic parasite count regardless of species, 5,000 randomly selected erythrocytes were counted in blood smears, and intraerythrocytic parasites were quantified (Megía-Palma et al 2020). All parasite counts were performed by the same observer to avoid observer bias. Data obtained from intraerythrocytic parasites were evaluated as parasite prevalence and intensity (Amo et al 2005b). Parasite prevalence was determined according to sexes as the percentage of lizards infected with blood parasites. Parasite intensity was obtained for each individual as the number of red blood cells with intraerythrocytic parasite found in 5,000 cells. As the aim of the study was to compare overall parasite load between populations rather than to characterize parasite diversity, parasites were quantified collectively. For each individual, we determined the heterophile-to-lymphocyte (H/L) ratio, a well-established marker of physiological stress and inflammatory responses (Davis et al 2008).

The snout-vent length (SVL) of lizards was measured using a digital caliper (Mitutoyo Corporation, Japan; 0.01 mm), and body weight (BW) was determined using a digital scale (Weight Lab Instruments; 0.01 g). To calculate the body condition values of each lizard, the SMI (Scaled Mass Index) proposed by Peig and Green (2009) was used: $SMI = Mi \times (L0 / Li)^{bSMA}$. According to this equation, Mi represents the body mass (g), Li is the body length (mm) of the individual, L0 is the mean body length of the studied population, and bSMA is the scaling exponent of the regression between body mass and body length. Since the body condition of an individual may be affected by autotomy, autotomized individuals were excluded from the analyses. Sex was determined by examining the presence of hemipenis and femoral pores. Lizards were released back to their biotopes after analyses. The necessary permissions have been obtained from the Ethics Committee of Animal Experiments of Çanakkale Onsekiz Mart University (decision no: 2022/03-08) for the studies that carried out.

Statistical analyses were carried out in SPSS and R environment (v.2024.04.2+764; Posit Team 2024). In all cases, $p < 0.05$ value was considered statistically significant. Kolmogorov-Smirnov Test for normality was used in analysis of the data. To determine differences in mean values within and between populations, Student's *t*-test or Mann-Whitney U test was used. Fisher's Exact test was performed to assess whether sex and population type (sympatric and allopatric) influenced prevalence. To evaluate parasite prevalence in the two different populations, Wilson Score was calculated to estimate the 95 % confidence intervals for the real population prevalence. Spearman's correlation test was applied to examine the variables associated with parasite intensity and body condition (SMI), as well as the direction of these relationships. A General Linear Model (GLM) analysis was conducted to evaluate the effects of sex, population type (sympatric and allopatric), and their interactions with these variables on the dependent variables (parasite intensity and SMI).

Results

Intra-population comparisons conducted to detect sex-related differences in all parameters revealed a statistically significant difference only in Scaled Mass Index values ($t = 2.486$, df = 17.980, $p = 0.023$) between males and females in the sympatric population. In the allopatric population, significant differences were observed between males and females in BW ($t = 3.946$, df = 14.510, $p = 0.001$), SMI ($t = 6.226$, df = 18, $p = 0.000$), and H/L ratio ($t = -3.122$, df = 18, $p = 0.006$). No sex-related differences in parasite intensity were detected in either population (table 1).

Examination of blood smears revealed the presence of intraerythrocytic parasites in individuals from both

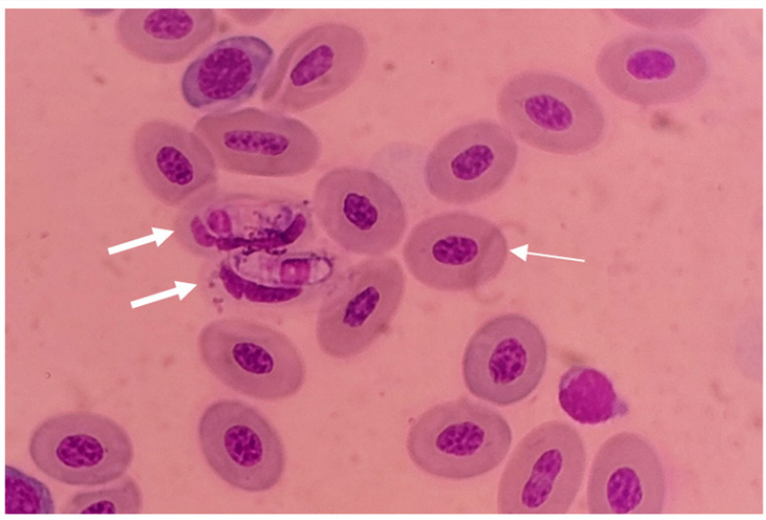

Fig. 2. Erythrocytes with (thick arrows) and without (thin arrow) intraerythrocytic parasites under a light microscope.

the sympatric and allopatric populations (fig. 2). In the sympatric population, parasite prevalence was calculated as 90% in males (95% CI = 66.13–98.91%) and 80% in females (95% CI = 55.49–94.78%), with no statistically significant relationship between prevalence and sex ($p > 0.05$, Fisher's Exact Test). In the allopatric population, prevalence was found as 70% in both males and females (95% CI = 41.33–91.63%), and no statistically significant relationship was detected between sex and prevalence ($p > 0.05$, Fisher's Exact Test).

To identify differences based on population type, males from the sympatric population were compared with males from the allopatric populations, and females from the sympatric population were compared with females from the allopatric populations.

There was no statistically significant relationship between parasite prevalence and population types (sympatric vs. allopatric) when comparing populations ($p > 0.05$, Fisher's Exact Test). Despite the high parasite prevalence in both populations, the mean parasite intensity was 25.50 ± 9.07 in sympatric males and 38.70 ± 18.40 in sympatric females. In the allopatric population, the mean parasite intensity was 5.70 in males and 4.70 ± 1.72 in females (fig. 3). When comparing parasite intensity between sympatric and allopatric individuals, statistically significant differences were detected between both males ($U = 20.500$, $Z = -2.239$, $p = 0.025$) and females ($U = 23.000$, $Z = -2.060$, $p = 0.0039$). Correlation analyses revealed a strong positive relationship between parasite intensity and both SVL ($r = 0.806$, $p = 0.005$) and BW ($r = 0.770$, $p = 0.009$) in sympatric males. Similarly, in allopatric males, parasite intensity showed a strong positive correlation with SVL ($r = 0.708$, $p = 0.022$). No correlations were detected between parasite intensity and other parameters in females from either population.

The results of the Scaled Mass Index (SMI), which reflects body condition (fig. 3), showed that sympatric males had a mean value of 4.54 ± 0.54, which was similar to the mean value of allopatric males (4.55 ± 0.49). In contrast, sympatric females had a mean SMI as 3.94 ± 0.52, whereas allopatric females had a lower value as 3.50 ± 0.19.

When comparing SMI values between sympatric and allopatric populations, statistically significant differences were found only in females ($t = -2.494$, $df = 11.457$, $p = 0.0029$). Results from the Spearman correlation analysis indicated a strong negative relationship between SMI and the H/L ratio in sympatric females ($r = -0.758$, $p = 0.011$), whereas no significant correlations were found in allopatric females.

When comparing populations in terms of H/L ratio, an indicator of stress, no statistically significant differences were found between males ($p = 0.970$) and females ($p = 0.174$) in either sympatric or allopatric populations.

The results of the GLM analysis (table 2), conducted to evaluate the effects of sex, population type (sympatric vs. allopatric), and their interactions on the dependent variables (Parasite Intensity, SMI), revealed that only sex had a significant effect on SMI ($F(1, 36) = 31.543$, $p < 0.001$). However, population type ($p = 0.149$) and the interaction between sex and population type had no significant effect on SMI ($p = 0.130$). For parasite intensity, the analysis indicated that population type had a statistically significant effect ($F(1, 36) = 6.760$, $p = 0.013$), whereas sex ($p = 0.559$) and the interaction between sex and population type had no significant effect ($p = 0.497$).

Discussion

In natural populations, there are numerous potential indicators showing that parasites affect the overall health of their hosts. These include a low body condition index (Stevenson and Woods Jr 2006), intense endo- and ectoparasitic infections (Oppliger et al 1999), and weaker immune status (Møller et al 1998). Although the prevalence and intensity of parasitic infections in reptiles have significant detrimental effects on host ecology and evo-

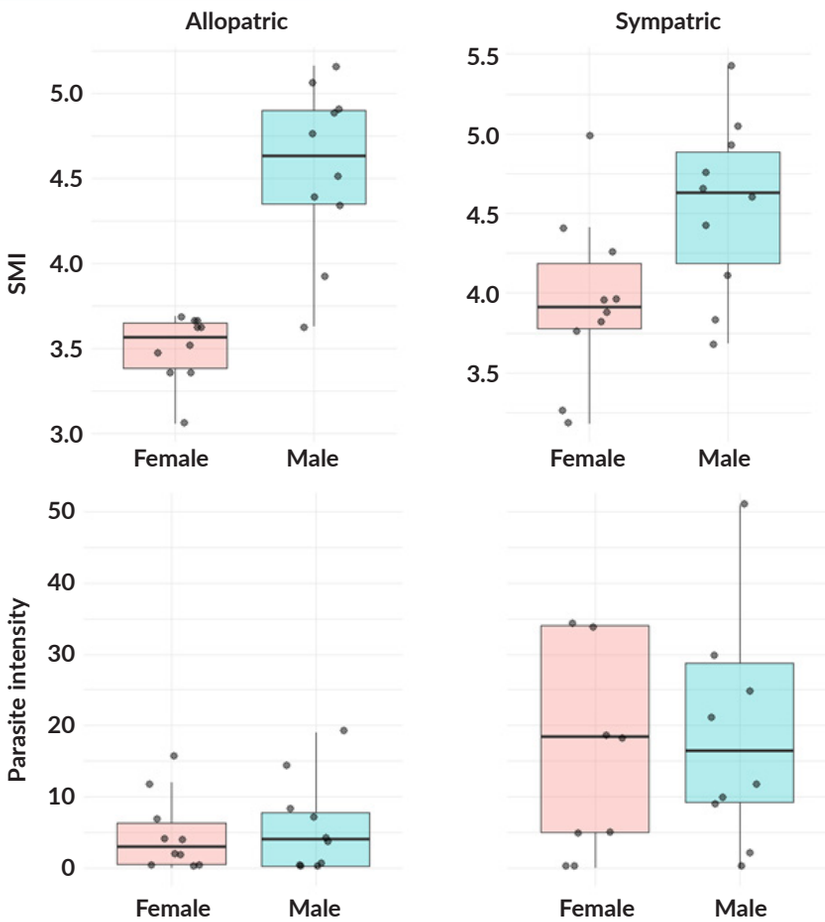

Fig. 3. Parasite intensity and body condition (Scaled Mass Index) across sympatric and allopatric populations, categorized by sex. Boxplots show median and interquartile range, with individual values overlaid as points.

lution in many aspects, they are still not well understood (Smallridge and Bull 2000, Eisen 2001, Amo et al 2005b).

Our findings indicate that parasite intensity in *P. muralis* individuals from the sympatric population was statistically higher than that in individuals from allopatric population, as expected. While the body condition of females in the sympatric population was higher, no significant relationship was found between parasite intensity and body condition or H/L ratios. However, a statistically significant positive correlation was detected between parasite intensity and body size in males from both populations. Furthermore, in *P. muralis*, population type (sympatric or allopatric) was identified as the most influential factor on parasite intensity, whereas sex was the most influential factor on body condition.

The prevalence and load of blood parasites vary significantly depending on numerous biotic and abiotic factors, such as host phenology, activity patterns, hormone levels, vector ecology, factors related to the host immune system under different environmental conditions, precipitation, temperature, and anthropogenic influences (Schall and Marghoob 1995, Schall and Pearson 2000, Storniolo et al 2022). According to our results, no statistically significant relationship was found between parasite prevalence and either sex or population type (sympatric vs. allopatric) ($p > 0.05$, Fisher's Exact Test). However, based on the mean values in our findings, individuals from the sympatric population showed a relatively higher prevalence percentage compared to individuals from allopatric population. Similarly, a study on two lacertid species (*Iberolacerta horvathi* and *Podarcis muralis*) concluded that syntopy and allotopy had no statistically significant effect on parasite prevalence (Dajčman et al 2021). Another study on *Podarcis muralis* populations from Serbia compared prevalence values between five urban and five rural populations and found no statistically significant difference, though parasite presence exhibited variability among populations in both habitat types (Lazić et al 2017). While our study did not find a relationship between sex and prevalence, a study from Spain reported that the occurrence of ectoparasites and intraerythrocytic hemogregarines was higher in males of *P. muralis* (Amo et al 2005b). In contrast, a study on a *P. muralis* population from Italy found no statistically significant sex-related difference in parasite prevalence (Storniolo et al 2019). Findings from studies on *P. muralis* across various geogra-

Table 2. Statistical results obtained from GLM on individual parasite intensity and SMI values with sex, population type (sympatric vs. allopatric) and interaction effects. * Population type examines the combined effects of sex and population type.

	df	Sum of Squares	F	*p*
SMI				
Sex	1	6.823	31.543	**< 0.001**
Population type	1	0.471	2.177	0.149
Sex* population type	1	0.520	2.403	0.130
Parasite Intensity				
Sex	1	372.100	0.348	0.559
Population type	1	7236.100	6.760	**0.013**
Sex* population type	1	504.100	0.471	0.497

phical regions suggest that parasite prevalence may vary based on different abiotic and biotic factors.

Although both populations exhibited high parasite prevalence, we found significant statistical differences in parasite intensity between sympatric and allopatric populations. While sex had no effect on parasite intensity, population type was identified as a determining factor. Individuals from the sympatric population showed significantly higher parasite intensity in both sexes when compared with individuals from the allopatric population. Although studies examining the effect of population type on parasite intensity in lizards are limited, Dajčman et al (2021) investigated parasite-host relationships between *Iberolacerta horvathi* and *Podarcis muralis* in syntopic and allotopic habitats. They found that parasite intensity was higher in syntopic populations. Additionally, in *P. muralis*, males had higher prevalence and intensity than females. Similar to the findings of our study, Dajčman et al (2021) reported that the mean parasite intensity in syntopic *P. muralis* was 5.39 for males and 2.18 for females, whereas in allotopic individuals, it was 3.50 for males and 0.91 for females. The *P. muralis* individuals examined in our study were ecologically similar enough to *P. tauricus* to compete for the limited resources available in their sympatrically shared habitat (Baycan et al 2024). Ectoparasites such as mites may contribute to the transmission of blood parasites among closely interacting lizards (Godfrey et al 2006). It is hypothesized that one of the factors contributing to the higher blood parasite intensity observed in the sympatric individuals in our study may be this. In addition to the close contact observed during competition, lizards, despite not forming social groups, share shelter sites and hibernacula, which may facilitate the transmission of blood parasites via mite vectors.

Although no significant difference was observed between sexes within populations in terms of parasite intensity, based on the mean values, females in sympatric population (38.70 ± 18.40) and males in the allopatric population (5.70 ± 2.05) showed higher values. Previous studies have generally found that males have higher parasite intensity than females (Dajčman et al 2021, Megía-Palma et al 2024). However, some studies report that parasite intensity in females tends to be higher in the spring (Amo et al 2005b). During the reproductive season, elevated testosterone levels in males lead to increased aggressive behaviors (Amo et al 2005b). As a result, males frequently interact with their conspecifics not only during mating but also during fights with other males. Additionally, males with higher testosterone levels exhibit more active behavior, which increases their encounters with females that may act as parasite vectors (Olsson et al 2000, Megía-Palma et al 2024). Supporting these hypotheses, individuals with higher interaction rates with infected individuals may show increased blood parasite intensity.

Furthermore, we found a positive relationship between parasite intensity and body size in males from both populations, indicating that larger individuals tend to harbor more parasites. Lazić et al (2017) similarly reported that SVL (snout-vent length) influenced parasite load, suggesting that larger individuals had higher parasite intensity. This pattern, frequently observed in lizard studies (Oppliger et al 1999, Amo et al 2004), supports the hypothesis that larger lizards may have encountered more parasites as they age, and their lifespans extend (Lazić et al 2017).

Our results on body condition (SMI) showed no statistically significant differences between sexes, though males in both populations exhibited higher body condition values. However, in our study, female individuals from the sympatric population had significantly higher body condition than those from the allopatric population. But no significant relationship was detected between parasite intensity and body condition. This finding suggests that individuals may have developed a certain tolerance to intraerythrocytic parasites. A review of previous studies has revealed inconsistent findings regarding the relationship between body condition and blood parasite infections. While some studies on different lizard species found no correlation between body condition and parasite prevalence or intensity (Amo et al 2005a, Damas-Moreira et al 2022), others reported a positive correlation (Molnár et al 2013, Maia et al 2014). A study on *P. muralis* found that individuals with higher blood parasite intensity had better body condition (Amo et al 2005b), whereas the opposite was documented for insular *P. lilfordi* (Garrido and Pérez-Mellado 2013). Increased activity in lizards with better body condition may lead to more competitive encounters, increasing their risk of infection via hematophagous vectors (Amo et al 2005a, Garrido and Pérez-Mellado 2013). Additionally, it has been stated that lizards with better body condition may tolerate high parasite loads (Comas 2019). However, if heavily parasitized individuals with low body condition have higher mortality rates, parasite-carrying individuals may exhibit better body condition compared to those without parasites (Amo et al 2005a). Variability in body condition results may also stem from the limited accuracy of different body condition indices used (Peig and Green 2010), complicating the relationship between parasite load and body condition across studies.

Recent studies have evaluated parasite load in reptiles using immune markers, particularly H/L ratios (Sperry et al 2009, Davis and Maney 2018). Although our findings showed slightly higher H/L values in the sympatric population, the highest value was unexpectedly recorded

in allopatric females. Additionally, the increase in body condition of sympatric females was negatively correlated with the stress indicator H/L ratio, suggesting that as stress factors such as parasitic infections increase, body condition value may decline. Contrary to our findings, a study on a lacertid species found that red mite infection led to a decrease in heterophil ratios, a reduction in H/L ratio, and a decline in body condition index (Hernandes-Córdoba et al 2019). However, studies indicate that the relationship between parasite load and H/L ratio is complex (Davis and Maney 2018).

In conclusion, our study on *P. muralis* indicates that parasite intensity is related to population type, while body condition is influenced by sex. Our findings do not support the assumption that intraerythrocytic parasite-infected reptiles necessarily exhibit lower body condition. Moreover, we emphasize the need for more precise methods to assess immune responses and body condition and the use of advanced genetic tools to better understand the effects of blood parasites on hosts. We recommend comprehensive studies integrating different body condition indices and ecological factors to clarify the relationship between parasite load and body condition.

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