

**Title: Sex-specific effects of infection in an island endemic wildlife species.**

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## **ABSTRACT**

Presence and impacts of pathogen infections in wildlife are known to be determined by a hierarchical set of factors including host, habitat, and general environment characteristics. While an increased number of studies have been documenting host-parasite interactions in natural systems, little is known about these impacts in island ecosystems. To elucidate the impacts of infection in island populations, we investigate the effects of *Hepatazoon* (and apicomplexan hemoparasite) wall lizard (*Podarcis erhardii*) populations, on 17 Cycladic islands (Aegean Sea, Greece). Specifically, we evaluated the effects of infection on multiple aspects of lizard life history from lizards from these islands. We found significant declines of condition in infected populations, though only in male lizards, suggesting a sex-specific response to infection. Mixed model analyses also suggest that some island level characteristics can affect the health and locomotion of lizards. These results indicate variability in the impacts of malarial infection across populations of *P. erhardii*.

***KEYWORDS: MALARIA, ISLAND, LIZARDS, HEPATAZOON***

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## TABLE OF CONTENTS

<b>Abstract</b>	<b>i</b>
<b>Acknowledgements</b>	<b>ii</b>
<b>Introduction</b>	<b>1</b>
<b>Methods</b>	<b>5</b>
<b>Results</b>	<b>9</b>
<b>Infection Status and Host Condition (BMI)</b>	<b>9</b>
<b>Locomotion</b>	<b>10</b>
<b>Blood Characteristics</b>	<b>11</b>
<b>Island Level Effects on Health Condition</b>	<b>11</b>
<b>Discussion</b>	<b>13</b>
<b>Health Condition Declines With Infection</b>	<b>13</b>
<b>Immune System Characteristic Assessment</b>	<b>17</b>
<b>Effects of Location on Infection Outcomes</b>	<b>17</b>
<b>Conclusion</b>	<b>19</b>
<b>Images</b>	<b>21</b>
<b>Tables</b>	<b>28</b>
<b>Appendix</b>	<b>34</b>
<b>References</b>	<b>35</b>

## INTRODUCTION

Parasitic organisms are ubiquitous across ecosystems, with some estimates suggesting that 40% of the world's animals are parasitic (Dobson *et al.*, 2008). Given their wide distribution, one can expect that parasites play a significant role in ecosystems through both direct and indirect host interactions (Hatcher *et al.*, 2006). Indeed, recent research has found that parasites are influenced not just by the physiology of their host (Tschirren *et al.*, 2007) but also by the prevailing environmental factors (Laurance *et al.*, 2013). Like other organisms, habitat conditions influence a parasite; such conditions such as temperature have influenced parasite development (Tinsley *et al.*, 2011). Thus, to fully understand the impacts of parasites on their hosts, we need to consider the prevailing environmental and ecological conditions

Parasites have evolved a wide range of life history patterns and virulence levels that translate into differing effects on hosts. Furthermore, infection with even closely related pathogens can have widely divergent symptoms on their hosts (Gilman *et al.*, 2007). Clear examples of these impacts include reductions in survival or reproductive fitness (Loker and Hofkin, 2015). However, not all parasites affect host survivorship directly; more often infection with parasites results in sublethal symptoms in their host necessitating the use of the other metrics beyond host mortality (Boots and Norman, 2000).

In reality, infection isn't relegated to one host, but affects multiple members of a population. If these infection impacts are widespread, we even see changes to a population or community (Wood *et al.*, 2007). By examining the impact of infection on a host, we predict how infections might affect other potential hosts in the population. One

such key measure that has emerged over the years to assess host condition is body mass index (BMI) (Jakob *et al.*, 1996). Such an index considers the mass of an animal corrected for frame size and is analogous to human BMI, representing the amount of resources and caloric reserves available to that individual (Jakob *et al.*, 1996). Such resources typically in the form of adipose tissue are particularly important in strongly seasonal environments where food may be scarce for many months of the year (Fantuzzi and Braunschweig, 2014). Additionally, an organism's locomotory performance, such as stamina or sprint speed, is often assessed by wildlife ecologists. It helps scientists determine not just an individual's ability to navigate its environment but also to compete for mates (Husak *et al.*, 2008) and most importantly to escape predation (Srygley and Dudley, 1993). Locomotory performance traits have therefore been linked to other traits, such as survivorship (Taylor and McPhail, 1985) and reproductive fitness (Wauters *et al.*, 1995). Lastly, impacts of infection on host can be assessed by quantifying both endurance or bursts of sprinting (Schall, 1982). As a result, in order to evaluate the full spectrum of impacts of parasitism on its host, it is important to investigate a multiple of traits.

Many studies investigating host-parasite interactions in nature have done this in mainland settings (Brunner and Eizaguirre, 2016; Turner *et al.*, 2021). In comparison we only have a comparatively poor understanding of host-parasite interactions in an island system, especially when it comes to impacts on host physiology. The limited disease ecology work conducted in island systems suggests that parasitic organism diversity declines with island size, presumably reflecting the diminished host populations available for infection (Nieberding *et al.*, 2006). In addition, some evidence suggests that parasites with simple (direct) lifecycles may survive better in smaller fragments (Roca *et al.*,

2009). Multiple processes occur in island systems that have the potential to affect host-parasite interactions, often in opposite directions. For example, declining host population size typically will result in reduction in a host's genetic diversity (Frankham, 1998; Acevedo-Whitehouse *et al.*, 2003; Suzán *et al.*, 2012; Santonastaso *et al.*, 2017). This in turn is expected to attenuate the hosts' immune capacity as well as the concomitant ability to resist parasitism (Spielman *et al.*, 2004; Whiteman *et al.*, 2006; Luikart *et al.*, 2008). Of course, the same isolation processes undermining host genetic diversity may impact the parasite, resulting in reduced ability to infect the host population (Roca *et al.*, 2009). Furthermore, it is presently not clear how habitat isolation will affect parasite virulence and impact on host fitness. As a result, it is clear that habitat fragmentation can influence several aspects of parasite ecology and lead to divergent outcomes making ecological predictions difficult (Foufopoulos *et al.*, 2022).

Examining islands offers insight into the long-term effects of habitat fragmentation on parasitism. Islands can serve as a microcosm for other isolated ecosystems; its relatively undisturbed nature allows us to examine patterns of evolution undisturbed, which we then can use to create general models (Whittaker *et al.*, 2017; Watson, 2019). Given the rise in human caused habitat reduction, it is essential to understand how these changes will impact ecosystems in the long term (Haddad *et al.*, 2015).

The Aegean Sea (Mediterranean Sea, Greece) encompasses over 2,000 land-bridge islands, most of which became separated from nearby landmasses by rising sea levels following the end of the last ice age (Poulos *et al.*, 2009). Consequently, populations of the Aegean wall lizard (*Podarcis erhardii*) (Figure 1), a species with low



dispersal abilities (Foufopoulos and Ives, 1999; Hurston *et al.*, 2009), became isolated on different islands (Fornberg, 2017). These islands vary tremendously with size, with some resembling mainland areas, while others being little more than rocks that harbor halophytic vegetation (Fornberg, 2017). Recent genetic analyses have revealed not only that *Podarcis* population size and genetic diversity roughly scale with island size, but also that there is a lack of significant genetic exchange between island populations (Hurston *et al.*, 2009; Santonastaso *et al.*, 2017).

*Podarcis* lizards are parasitized by a broad diversity of parasitic taxa, including gastrointestinal helminths, mites, ticks, and apicomplexan hemoparasites (Roca *et al.*, 2009; Pafilis *et al.*, 2013; Fornberg, 2017; Foufopoulos *et al.*, 2017). In particular, *Podarcis* lizards are often hosts to malarial-like parasites in the genus *Hepatozoon* (Oppliger *et al.*, 1998; Fornberg, 2017). *Hepatozoon* is known to infect a wide range of hosts including mammals, birds and reptiles (Smith, 1996). This parasite appears to be most often transmitted by vectors such as mites, though alternative transmission pathways may be involved (Smith, 1996). The few studies that have explored *Hepatozoon* infections in *Podarcis* have found that infections can range from being asymptomatic to significant reductions in host body mass index (BMI) (Moreira, 2013; Garrido and Pérez-Mellado, 2013); this variation in health suggests substantial variation in impacts of infection across populations. However, little is known about the occurrence and effects of *Hepatozoon* in *P.erhardii* lizards. Recent research has found that *Hepatozoon* prevalence was higher in younger islands and in islands with denser host populations (Fornberg, 2017). Overall, these findings suggest that *Hepatozoon* infections, in this species at least, are affected by the process of habitat fragmentation, as well as the

biotic and abiotic characteristics of an island and set the stage to investigate interisland variation in infection impacts on hosts (Fornberg, 2017).

Little information exists at present on the effects of hemogregarine parasites, including *Hepatozoon*, on lizard hosts. Additionally, the effect of island variation on disease and host health is not well understood. Based on basic epidemiological theory, we hypothesize that *Hepatozoon* infection impacts on the hosts will depend on a variety of host, population and island characteristics and will test these by comparing infected and uninfected lizards across islands (Hurston *et al.*, 2009; Fornberg, 2017). More specifically, we predict that infection impacts on hosts (as measured in body mass index, stamina, running speed, and immune system function) will depend on intensity of infection, with more heavily parasitized lizards experiencing more adverse health impacts (Garrido and Pérez-Mellado, 2013; Fornberg, 2017). Furthermore, we predict that host impacts will depend on previously studied island characteristics, such as island area, island age, predators, and density (Fornberg, 2017; Semegen, 2018). This study offers for the first time the opportunity to understand how impacts of infection with a common parasite varies across an archipelago system.

## **METHODS**

To determine the nature of host-parasite interactions between *Hepatozoon* and its lizard hosts in an island setting, we investigated Aegean wall lizards from numerous 17 islands across the Aegean Sea (Figure 2). These particular island populations were chosen because they were also included in previous studies that had examined the lizard genetic diversity (Hurston *et al.*, 2009) as well as the ecological distribution of *Hepatozoon* across island ecosystems (Fornberg, 2017).

We visited all study island in the period between May and July 2016, and we captured lizards via nooses attached to telescoping poles (Fornberg, 2017). Collection occurred between 9:00 AM and 5:00 PM, corresponding with the lizards' main period of activity (Fornberg, 2017). Captured animals were transported to the laboratory where they were maintained in species-appropriate terraria until morphological measurements, blood sample collection, and locomotor performance assays were completed (Semegen, 2018). Overall, 18-34 lizards were collected from each island, and all animals were returned to the site of capture following immediate completion of the study (Semegen, 2018). We obtained several morphological measurements including mass (in *g*), as well as size (snout-vent length-SVL), hind leg length, and head height, width and length (all in *mm*) (Donihue, 2016; Donihue *et al.*, 2016). Body mass was regressed on SVL separately for males and females; the residuals were used to determine a obtain body mass indices for each individual in this species (Jakob *et al.*, 1996).

To assess locomotory ability, we measured stamina and sprint speed for a subset of 294 and 459 lizards, respectively. To measure stamina, 20 lizards from each island were encouraged to run around a circular, sand-covered racetrack (230 cm in diameter and 40 cm height) after thermoregulating for one hour (Semegen, 2018). Each lizard's temperature was then recorded at the end of the hour (Semegen, 2018). Animals were then allowed to run at a slow to moderate pace until they failed to respond to a standard tactile stimulus (light tapping at the base of the tail), at which point duration of movement was recorded; these stamina trials were conducted 3 times for each lizard, with highest values being recorded (Semegen, 2018). To quantify sprint speed, lizards were raced down a 2.71 meter long racetrack at which time a dorsal view of the lizard was recorded

via a high-speed video camera at 240 frames per second (fps) (Donihue, 2016). This was repeated 3 times and highest values being recorded (Donihue, 2016). These videos were analyzed using specialized software SAVRA and maximum sprint speed was determined via splined calculations by identifying the maximum segment of the racetrack covered per 5 frames (Donihue, 2016).

We sampled *P. erhardii* blood to determine the presence and characteristics of *Hepatozoon* infections. To avoid any possible interference, we did not conduct blood sampling until all locomotor performance measurements were concluded (Semegen, 2018). A small droplet of blood was drawn from a cut toe and applied as a thin smear to a microscopy slide using standard procedures (Schall, 1990b). Slides were air-dried and then preserved with methanol and stained with Giemsa to aid in *Hepatozoon* identification (Telford, 2008). Each slide was scored using a compound microscope under 1,000x magnification, with 10,000 red blood cells (RBCs) evaluated in each slide (Fornberg, 2017). *Hepatozoon*-infected host erythrocytes are readily recognized by their enlarged, distorted shape and displaced nuclei (Telford, 2008; Fornberg, 2017). Parasite load (parasitemia) was expressed as number of infected cells per 10,000 RBCs (Fornberg, 2017). Additionally, we distinguished animals as either light or heavily infected based on whether parasitemias were above or below the median value for the population (19 infected cells/10,000 RBCs).

To understand physiological changes in an animal's blood, we also determined the relative abundance of the primary white blood cell populations (lymphocytes, basophils, eosinophils, heterophils, monocytes, and thrombocytes) (Campbell, 2015). This was accomplished as number of cells per 10,000 RBCs scored (Fornberg, 2017).

Previous studies have used white blood cell profiles to assess lizard immune responses to hematological parasites (Motz *et al.*, 2014).

Island characteristics (Table 1), such as size and age, were gathered from prior studies (Foufopoulos and Ives, 1999; Hurston *et al.*, 2009). Predator presence was calculated via the presence/absence of 6 distinct predator categories on each island; these were classified by predation styles (resulting in rats, boas, birds, vipers, other snakes, and mammals) (Semegen, 2018). We calculated lizard density on each island, noting the number of observations of lizard activity throughout 100 m long, 5 m wide transects (Brock *et al.*, 2015; Semegen, 2018). Because the study islands were a subset of a much larger population, island characteristic variables were treated as a random effects; these included island identity, island age, area, lizard density, as well as number of resident predators (Fornberg, 2017; Semegen, 2018).

Statistical analyses were conducted to compare the health and condition of lizards across different islands and infection frequency using R (R Core Team, 2020). Values of parasitemia, stamina, sprint trial and white blood cell measures were ln-transformed. Independent sample t-tests were conducted to determine if there was a significant difference in snout vent length between infected and uninfected lizards. Linear regressions of parasitemia against the response variables of BMI, stamina, and sprint speed were also generated.

To assess the effect of island characteristics on lizard condition, mixed models were developed (Bates *et al.*, 2015). With these models, we examined how models of BMI, stamina, and sprint speed were affected by the inclusion of island identity as a random intercept. In addition to the island variable, we also included factors such as sex,

parasitemia and infection status as fixed effects. To conduct analyses via the lme4 package in R, we generated both an initial null model and full model (Appendix: Equation 1), the key difference being the inclusion of island identity as a random factor; a likelihood ratio test assessed the difference in accuracy for these models (Bates *et al.*, 2015). Additionally, we compared the random intercept island model to a random slope model which contained the effect of island identity on parasitemia's slope (Winter, 2013).

## RESULTS

### Infection status and host condition (BMI)

We analyzed 460 *Podarcis erhardii* from 17 islands and found that 67.8% were infected with *Hepatozoon* (312 infected and 148 uninfected). Average infection rates did not differ between sexes (males 67.3% [187/278] versus females 68.9% [125/182]; chi-squared = 0.047,  $p = 0.829$ ). Likewise, there were no differences in the fraction of above median infections (parasitemia >19/10,000RBCs) between sexes (Males 48.7% [91/187]; Females 48.8% [61/125]; X-squared = 0.046505,  $p = 0.8293$ ).

There was no significant difference in parasitemia between sexes (Parasitemia<sub>m</sub>=62.743 ± 10.528/10,000 RBC; Parasitemia<sub>f</sub>=69.484 ± 12.86/10,000 RBC; n=460,  $t=0.7125$ ,  $P = 0.4766$ , independent samples t-test). There was also no significant difference in body size (SVL) between infected and uninfected lizards ( $t=0.349$ ,  $P=0.727$ ; n=460, independent samples t-test).

In females, there was no significant difference in of BMI between infected and uninfected individuals (n=182,  $t = 0.174$ ,  $P = 0.862$ , independent sample t-test) (Figure 3). While infection status was not significant with infection status, heavily infected

females showed a marginally lower BMI (n=125,  $t = 1.834$ ,  $P = 0.069$ ; independent samples t-test) (Figure 4). T-tests of BMI and infection show significantly lower BMI than uninfected male lizards (n=278,  $t = 1.834$ ,  $P = 0.051$ ) (Figure 3). Likewise, of male BMI and infection intensity was significantly lower in heavily infected lizards (n=182,  $t = 3.153$ ,  $P = 0.002$ , independent samples t-test) (Figure 4). Parasitemia and BMI linear regressions show a significant negative relationship in both males and females ( $r_M = -0.295$ ,  $P_M = 4.29e-05$ ;  $r_F = -0.234$ ,  $P_F = 0.009$ , linear regression) (Figure 5).

### **Locomotion**

I analyzed locomotory performance (stamina and sprint speed) in *P. erhardii* in relationship to the presence of *Hepatozoon*. In female lizards, stamina was significantly associated with infection status ( $t = -2.614$ ,  $P = 0.01$ , n=142, independent samples t-test). However, t-tests of high/low infection intensity and stamina were not significant for females ( $t = 0.2$ ,  $P = 0.842$ , n=142, independent samples t-test). In females, the linear negative relationship between stamina (ln transformed) and endurance was marginally non-significant ( $r = -0.199$ ,  $P = 0.0547$ , linear regression) (Figure 6).

In males, t-tests of infection status and stamina were not significant ( $t = -1.622$ ,  $P = 0.107$ , n=152, independent samples t-test). However, infection intensity was significantly related to stamina for infected males ( $t = 3.116$ ,  $P = 0.003$ , n=152, independent samples t-test), with animals carrying heavier infection showing less stamina. Linear regressions of stamina against parasitemia in infected lizards revealed a significant negative relationship of male stamina (ln transformed) to parasitemia (ln transformed) ( $r = -0.333$ ,  $P = 0.0006$ , linear regression) (Figure 6). In follow-up analyses,

the effect of parasitemia remained statistically significant, while other factors such as hind span did not have a significant effect on stamina (Table 2).

Sprint speeds were not significantly associated with infection status for all lizards ( $T_{M} = -1.68$ ,  $P_{M} = 0.0947$ ;  $T_{F} = 1.098$ ,  $P_{F} = 0.2736$ , independent samples t-test); speeds were significantly higher with higher infection intensity categories of males ( $t = -2.579$ ,  $P = 0.011$ , independent samples t-test), but not for female lizards ( $t = -0.1517$ ,  $P = 0.878$ , independent samples t-test). However, linear regressions of sprint speed, separated by sex against parasitemia in infected lizards were not significant ( $r_{M} = 0.099$ ,  $P_{M} = 0.178$ ;  $r_{F} = 0.05$ ,  $P_{F} = 0.585$ , linear regression).

### **Blood Characteristics**

We quantified the numbers of the six main types of blood cells (basophils, heterophils, lymphocytes, monocytes, eosinophils, and thrombocytes) among the different *P. erhardii* study populations (Table 3). Overall infected lizards showed small, but consistent reductions in population means relative to their uninfected counterparts for most immune cell types (basophils  $p = 0.010$ ; heterophils  $p = 0.019$ ; lymphocytes  $p = 0.013$ ; monocytes  $p = 5.11 \times 10^{-5}$ , independent samples t-test; see also Table 3, Figure 7). In contrast, there was a not significant difference between infected and uninfected hosts for eosinophils and thrombocytes (eosinophils  $p = 0.875$ ; thrombocytes  $p = 0.560$ ).

### **Island level effects on health condition**

To investigate the relationships between island level infection prevalence, as well as average infection intensity (i.e., average parasitemia) we estimated these values based on individual level data. Prevalence of infection was calculated for each island as the



percent of slides taken on which we detected *Hepatozoon*, with average parasitemia values being derived as averages for all parasitemia occurring on each island. On an island level basis, there is a significant relationship between parasitemia and prevalence ( $R^2=0.477$ ,  $b_1=21.475$ ,  $P=0.003$ ).

We found that inclusion of island identity in the mixed model comparative analysis increased model accuracy. Likelihood ratio tests between the initial null (without island level effects) and full models (with island level effects) of all three response variables (BMI, stamina, and sprint) were significant ( $P<0.05$ ) (Table 4, 5, and 6). However, comparisons between a random intercept and random slope model of island identity were not significant for BMI, stamina, or sprinting ( $P=0.086$ ,  $P=0.9985$ ,  $P=0.387$ , respectively) (Table 7, 8, and 9). Thus, we elected to use the random intercept model for our subsequent analysis.

The following likelihood ratio tests with more specific island factors (such as island age, lizard density, and the number of predators) varied in changes to model accuracy. The inclusion of island area in conjunction with island identity within our models created singular boundary errors in all 3 subsequent mixed models; inspection of this error with the rePCA function revealed that island area had a variance value of 0. However, this lack of variance disappeared when island identity is not included in the comparative mixed models, suggesting high covariance between island identity and area.

For BMI, only the inclusion of lizard density as a random variable improved model accuracy ( $P=0.019$ ) (Table 10). It should be noted that even with this inclusion, parasitemia as a fixed factor was still somewhat significant ( $P = 0.081$ ). Other variables, such as island age or predator numbers were not significant or resulted in redundancies

(Table 10). The inclusion of specific island factors did not improve the stamina mixed model (Table 10). Sprint speed models improved with the inclusion of predator variables ( $P = 0.03$ ); however, fixed effects, such as parasitemia were not significant ( $P=0.625$ ).

## **DISCUSSION**

### **Health condition declines with infection**

The overall aim of this study is to understand how hemoparasite infections affect their hosts and elucidate how variation in island environment may shape the outcome of host-parasite interactions. *Hepatozoon* infections were widespread in the study populations, with both males and females being equally infected. Presence of these hemoparasites had clear effects on various metrics of host condition including locomotory performance and BMI. However, these effects tended to be significant mostly in males and not in females. Furthermore, health and stamina showed a negative relationship with parasite load, again these patterns were most pronounced in males as well.

The body mass index (BMI) informs us of an individual's energy reserves (Schulte-Hostedde *et al.*, 2005); such assessments of condition have been linked to traits such as fitness (Wauters *et al.*, 1995). We found that infection with *Hepatozoon* had the potential to impact host BMI and that animals with severe infections had significantly lower body mass indices (Figure 3). Additionally, BMI declined as infection intensity increased (Figure 4). This is also reflected in linear modeling, with infected lizards BMI having an approximately linear relationship with ln transformed parasitemia (Figure 5). These results are consistent with earlier observations and demonstrate that lizard health

condition (such as fat reserves) can suffer with higher parasite load (Dunlap and Mathies, 1993; Dunlap and Schall, 1995). However, these patterns are not necessarily ubiquitous across lizards; other studies have found that more infected lizards had better body condition than their uninfected counterparts; in other words, sicker lizards were more fit than others (Amo *et al.*, 2005). Additionally, another member of *Podarcis* (*Podarcis vaucheri*) differed from *P. erhardii*, with no difference in body condition with increased parasitemia (Moreira, 2013). These differences across lizards suggest that impacts of high infection can vary significantly between species.

Multiple mechanisms are possible, through which parasite infection can impact a host's BMI. For example, infection may impact foraging behavior resulting in attenuated consumption of resources and concomitant reductions in BMI (Schall and Houle, 1992). This pattern may be exacerbated by reduced territoriality causing other animals to encroach onto an animal's territory, further reducing the resources available. This pattern was observed in the infection of *Sceloporus occidentalis*, another lizard species, which exhibited more submissive behavior when parasitized (Schall and Houle, 1992). Alternatively, infection with *Hepatazoon* may force the host to divert more resources towards upregulated immune function resulting in a higher energetic and nutrient investment towards immune defenses following infection (Rauw, 2012). Because most free-ranging animals operate under conditions of limited resource availability, this would force important trade-offs including reducing in adipose tissue reserves, therefore reducing infected lizard BMI (Coon *et al.*, 2016). Investigating the possible mechanisms linking infection with BMI would be a promising avenue of future investigations.

An organism's locomotor performance is associated with behavior such as territoriality (Sinervo *et al.*, 2000) or predator evasion (Miles *et al.*, 2001); both of these traits can inform an individual's fitness. In our study, infection intensity was significantly associated with locomotion in male lizards; however, this was only demonstrated in stamina (Figure 6). Previous work in other species has shown that malarial infection affects red blood cells counts and therefore impacts the ability of the blood to transport oxygen; this explains in turn the negative relationship between malaria infection and stamina (Schall, 1990b; Oppliger *et al.*, 1996). In contrast, our analyses did not find a relationship between parasitemia and sprint speed. Indeed, this mirrors similar results in past studies and is explained that sprinting is an anaerobic process nor affected by the capacity of blood to transport oxygen from the lungs to muscle tissue (Schall, 1982). As a result, it would be expected that hemogregarine infection would have less of an effect on this dimension of locomotory performance (Schall, 1990a).

Most of the impacts of infection, whether measured in BMI or locomotor performance, were more evident in male rather than in female lizards. These results, when considered together, suggest that in *P. erhardii*, *Hepatozoon* infection has an overall more severe impact on male lizards. At least two possible explanations may account for these sex differences. First, early studies had suggested that testosterone suppresses immune function (Belliere *et al.*, 2004) and therefore leads to more poorly controlled infections resulting in more severe impacts on the host. However subsequent studies have found little covariation between parasite loads and testosterone levels (Foufopoulos, 1999) suggesting instead that any mediating effect between male reproduction and parasitism may not be attenuated immune function, but rather altered

behaviors which increase exposure risk (Fuxjager *et al.*, 2011). Further contradicting the hormone hypothesis is that we did not find higher parasitemia in male lizards, as one might expect if they males failed to immunologically control *Hepatozoon* infections. Instead, the observed differences in the impacts of males versus females can be likely attributed to other differences in life history and reproductive schedule exacerbating the impacts of infection once it had been established. For example, male *Podarcis* typically display strong male-male aggression and territoriality (Edsman, 1989; Abalos *et al.*, 2016; Arakelyan *et al.*, 2019) behaviors which are both influenced by infection, and which amplify the negative impacts of parasitism (Salvador *et al.*, 1996). In *P. erhardii* specifically, the continuous agonistic challenges that males face primarily, may exacerbate the effects of *Hepatozoon* by preventing infected animals from foraging unencumbered (Donihue *et al.*, 2016). In contrast in females, any effects of infection are most likely to simply translate into reduced clutch size (Foufopoulos, 1999). Our results runs counter previous work, which has found more severe effects in females of other lizards, such as *Sceloporus occidentalis* (Schall, 1983, Foufopoulos 1999). The variability in the sex-specificity of infection suggests that outcomes of infection are not just shaped by reproductive schedule, but also that such schedules may vary widely depending on prevailing ecological conditions.

However, impacts of hemoparasite infection are not just limited to measures of body condition or locomotor performance. Instead, infection is expected to also result in potential reductions in survivorship or reproductive success. Such examples include the increase of predation following malarial infection or the reduction of reproductive output

in females (Schall, 1983; Møller and Nielsen, 2007). However, given the nature and scope of this study, we could not incorporate these measures into our analyses.

### **Immune system characteristic assessment**

*Hepatozoon* infection in *P. erhardii* resulted in significant reductions in circulating populations of basophils, heterophils, lymphocytes, and monocytes but not in eosinophils and thrombocytes. These results diverge from previous studies that have examined these effects of hemoparasites in other lizard taxa. For example, malarial infection induced higher lymphocyte populations in the lizard *Sceloporus occidentalis* (Motz *et al.*, 2014). However, other studies again have found variation of differences in white blood cell profiles; such examples can be seen in significant differences found in central Florida lizards (*Anolis carolinensis* & *Anolis sagrei*), while no such differences were detected in lizards from southwest Florida (Bessa *et al.*, 2020). This suggests substantial unexplained variability of immune defenses which may be attributable to characteristics of the host, the parasite taxon, or to characteristics of the prevailing environment. While infection might be expected to result in a stronger immune responses (Bain, 2017), infection can also suppress physiological activity, resulting in reductions in several other key metrics, such as hemoglobin production (Scholnick *et al.*, 2010).

### **Effects of location on infection outcomes**

On the scale of islands, the mixed model analyses reveal that island level characteristics can affect lizard health or locomotion, though not all island characteristics play the same role. Through this mixed model, we sought to understand how island characteristics affect lizard condition. The likelihood-ratio test suggests that the inclusion

of island level effects provided improved mixed models for BMI, stamina, and sprint trials. Examinations between random intercept models and the random slope model showed no significant change in model accuracy, suggesting that island characteristics are more directly correlated with response variables, rather than indirectly influencing response variables via changes to parasitemia (Winter, 2013).

Based on the results of the subsequent mixed model analyses, specific island factors such as predator numbers or lizard population density altered different models (sprinting and BMI, respectively) (Table 10). For the improvement with lizard density on BMI, it would be expected for higher density populations to live in higher quality habitats, which could then improve the lizard's BMI (Lunghi *et al.*, 2018). Hence, rather than being causally linked with each other, both elevated BMI and lizard population density may simply both be reflections of higher resource availability on some islands (Lunghi *et al.*, 2018). It should be noted that some significance of parasitemia on BMI did not fully disappear with the inclusion of lizard density, suggesting that even if environmental factors are included, infection retains its importance in explaining variation in BMI. However, while the inclusion of island identity improved stamina's mixed model, parasitemia in this model was no longer significant. Additionally, none of the examined island characteristics did not improve model accuracy; this suggests that other island characteristics affect the stamina of these lizards. Likewise, for the improved model of sprint speed, we did not see any significance with infection parameters, suggesting that predation accounts for more of the variation in this model, while parasitemia does not. This is consistent with earlier works on this system, which found sprint speeds (Semegen, 2018) were affected more by predator categories.

Thus, the inclusion of certain island factors can improve the accuracy of our model. However, with redundancies of island identity with other factors such as island area, we could not identify the functional connection between island identity and impacts of infection. Previous work in this system has found that occurrence of *Hepatozoon* was influenced by island characteristics: lizard populations on smaller and more isolated islands had higher rates of parasite prevalence (Fornberg, 2017). For example, there has been noted variation in the genetic diversity of hosts (whether lizards or birds) in islands, which could affect the parasitic defenses and health of populations – this is observed in both lizards and birds across islands (Whiteman *et al.*, 2006; Foufopoulos *et al.*, 2017). With this variation in mind, more work will need to separate island area from the island label, such as incorporating more islands of similar area measures.

## **Conclusion**

Our data indicate that infection with Hemogregarines clearly affects various metrics of condition and performance in *P. erhardii*. Specifically, infection with *Hepatozoon* affects *P. erhardii* through a reduction in both BMI and stamina, two functionally important aspects of life history. These effects, however, are less obvious in animals carrying light infections (i.e., with low parasitemia) and become more evident with heavier parasite burdens. Furthermore, we find that effects of infection on the host are dependent on host sex with males being more impacted than females even though they are not more frequently or more heavily infected. This suggests that other aspects of male life history such as male-male competition interact with, and then exacerbate the impacts of infection on the host. Additionally, there is evidence that island-based characteristics affect the outcome of infection in these lizards. All of these findings



suggest that outcome of infection is a complex result of sex and prevailing environmental conditions, including island location.

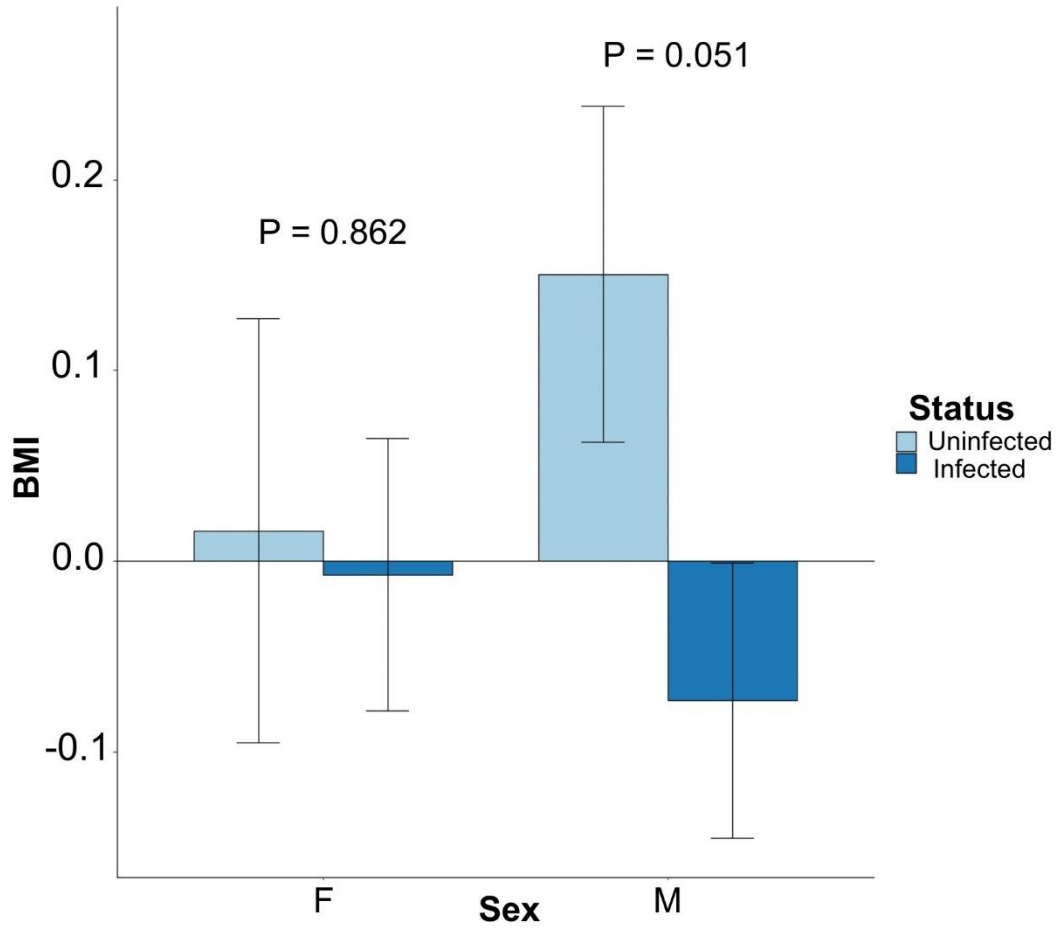
## Images



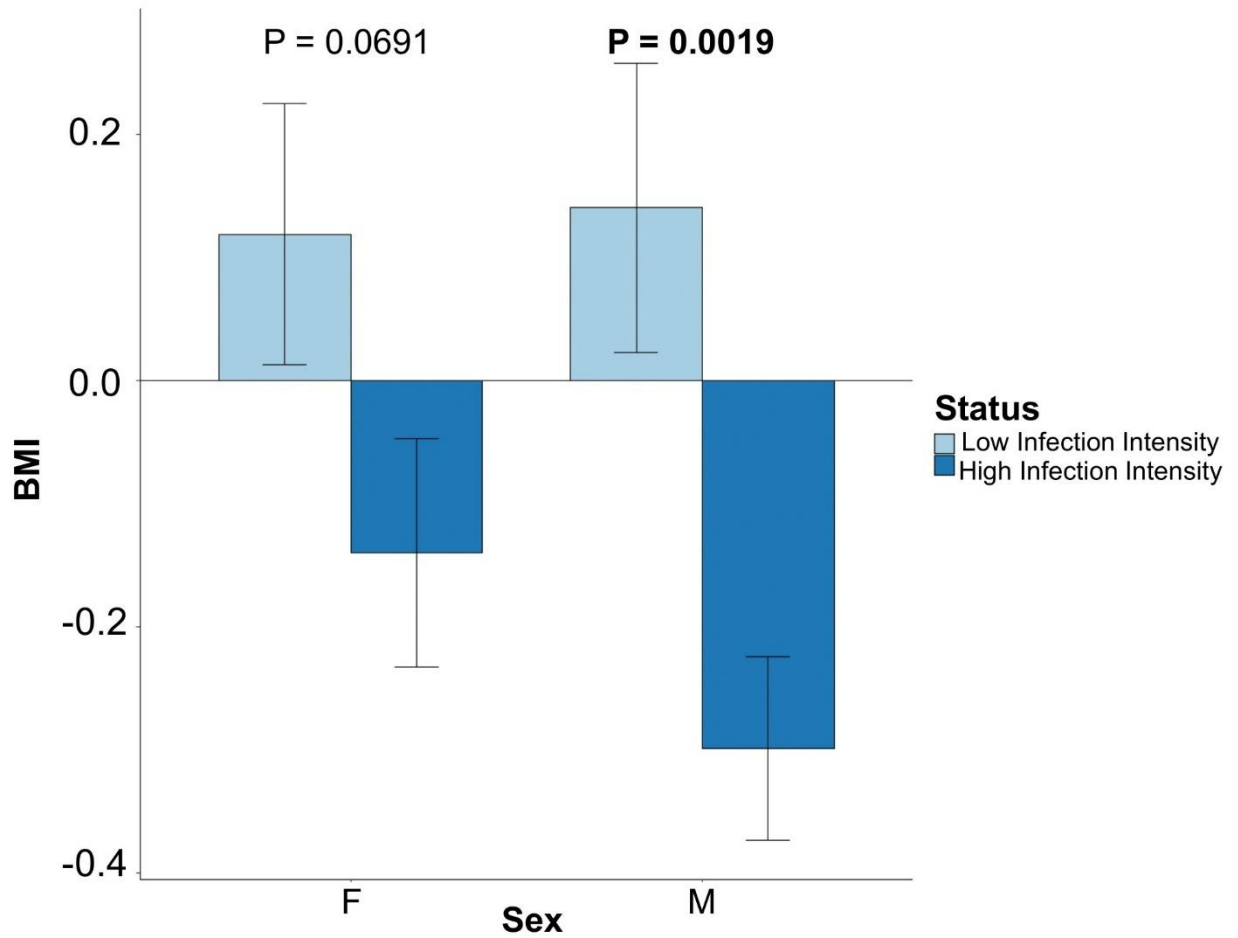
**Figure 1: Aegean Wall Lizard (*Podarcis erhardii*) (Foufopoulos, n.d.)**



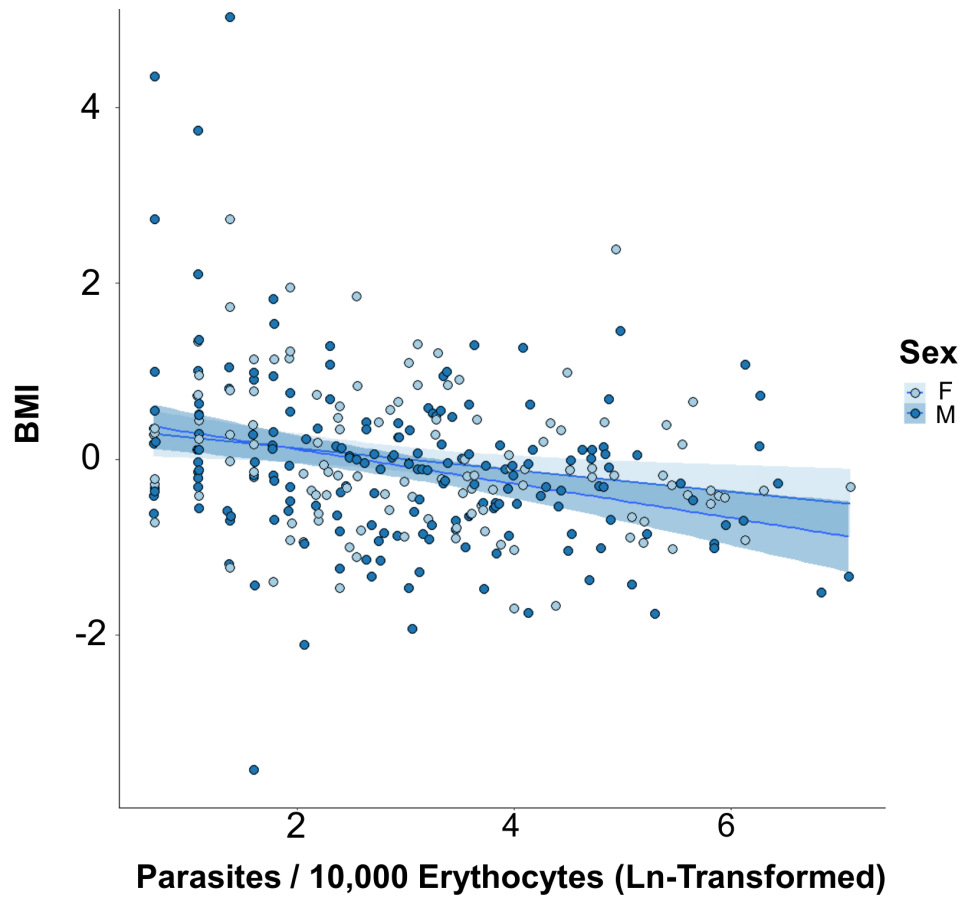
**Figure 2: Aegean Sea islands. Orange coloration signifies that a lizard population was gathered from an island (Main map: Ministry of the Interior and Administrative Reconstruction & inset map: ESRI)**



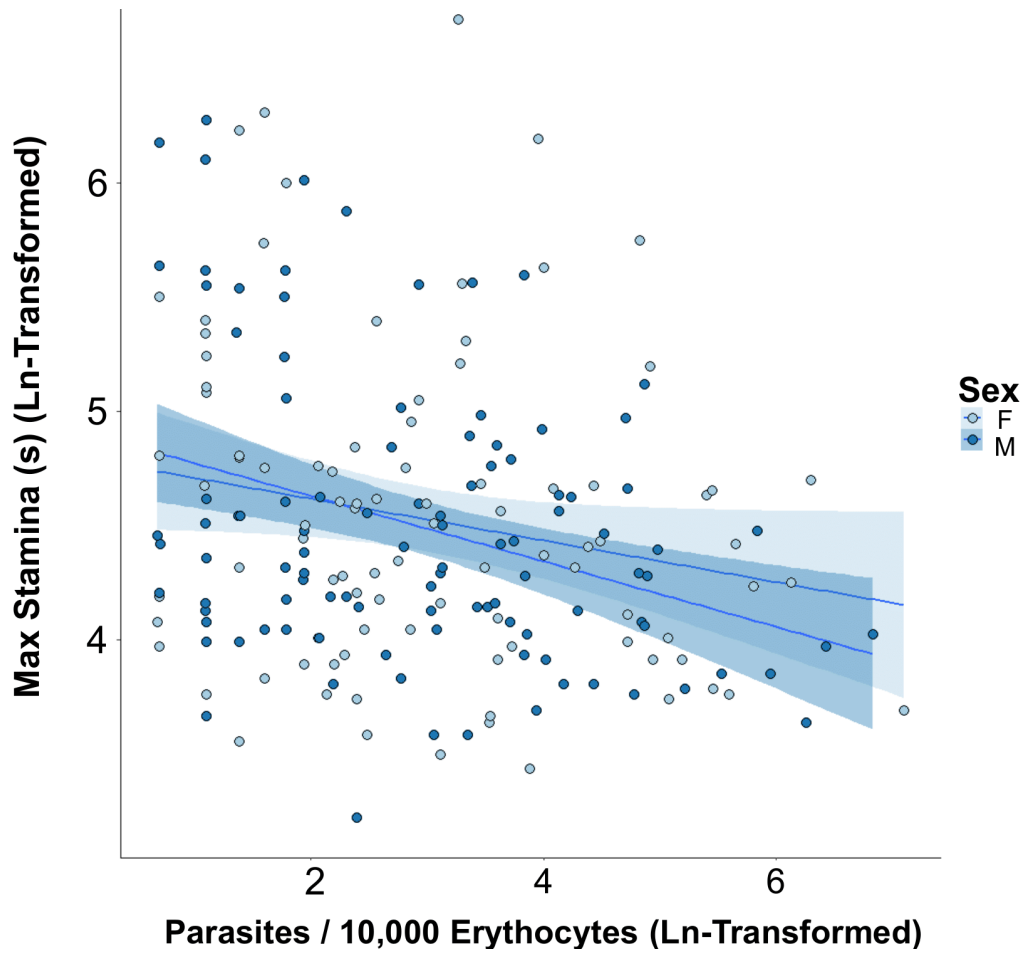
**Figure 3: Body Mass Index (BMI) of infected and uninfected lizards (separated by sex; M = male lizards, F = female lizards). This figure suggests a difference in infection status and BMI but only in male lizards.**



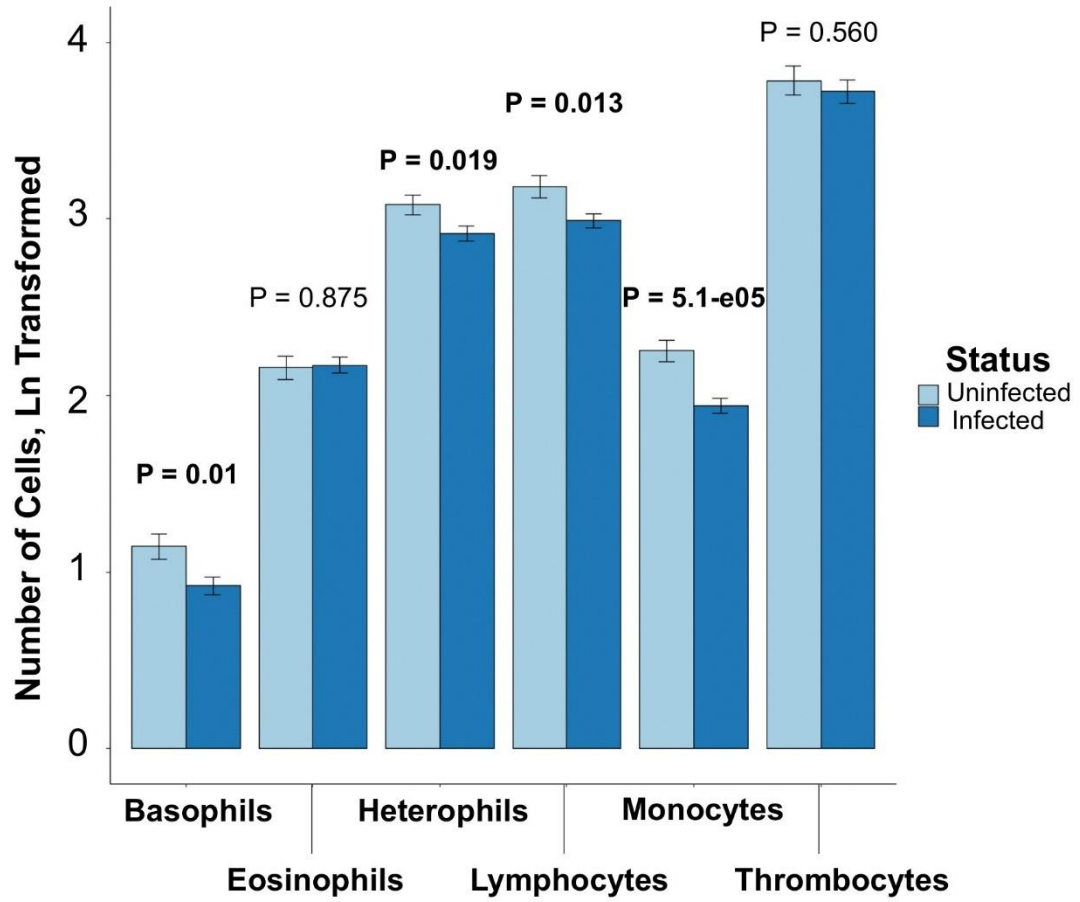
**Figure 4: Body Mass Index (BMI) comparing low and high infection intensity lizards (separated by sex; M = male lizards, F = female lizards). This figure suggests a difference in infection intensity and BMI but only in male lizards.**



**Figure 5: Body Mass Index (BMI) vs parasitemia for all infected specimens (separated by sex; M = male lizards, F = female lizards). ( $b_{1,M} = -0.195$ ,  $R^{2,M} = 0.087$ ,  $P_M = 4.29e-05$ ;  $b_{1,F} = -0.123$ ,  $R^{2,F} = 0.055$ ,  $P_F = 0.009$ ). This model suggests a decline in lizard BMI as infection intensity increases.**



**Figure 6: Max Stamina vs Parasitemia (separated by sex; M = male lizards, F = female lizards). ( $b_{1,M} = -0.143$ ,  $R^{2,M} = 0.111$ ,  $P_M = 0.001$ ;  $b_{1,F} = -0.091$ ,  $R^{2,F} = 0.04$ ,  $P_F = 0.054$ ). This suggests a decline in stamina as infection intensity increases, though stronger effects are in male lizards.**



**Figure 7: Comparisons for infection status (uninfected or infected) on blood characteristics. 4 of the 6 characteristics are significantly lower when infected (Basophils, heterophils, lymphocytes, and monocytes).**



<b>Island</b>	<b>Island Age (years)</b>	<b>Island Area (km<sup>2</sup>)</b>	<b>Lizard Density (Indv/ 100 m)</b>	<b>Predators Encountered</b>	<b>Male Infection Prevalence</b>	<b>Female Infection Prevalence</b>
<b>Amorgos</b>	200000	123	4	5	23.53% (4 of 17)	20.00% (1 of 5)
<b>Anafi</b>	5000000	40.37	8	3	94.74% (18 of 19)	69.23% (9 of 13)
<b>Andros</b>	5800	379.95	2	6	82.35% (14 of 17)	75.00% (3 of 4)
<b>Ano Antikeros</b>	15150	1.05	1	3	4.76% (1 of 21)	11.11% (1 of 9)
<b>Fidoussa</b>	600	0.63	6	3	54.17% (13 of 24)	42.86% (3 of 7)
<b>Glaronissi</b>	5600	0.16	3	2	62.50% (10 of 16)	57.14% (4 of 7)
<b>Ios</b>	11750	109.03	2	6	36.36% (4 of 11)	NA (0 of 0)
<b>Iraklia</b>	9800	18.08	4	6	27.78% (5 of 18)	28.57% (2 of 7)
<b>Keros</b>	9150	15.05	5	3	76.92% (10 of 13)	72.22% (13 of 18)
<b>Kisiri</b>	5700	0.01	2	2	93.75% (15 of 16)	100% (12 of 12)
<b>Lazaros</b>	9100	0.01	13	0	78.26% (18 of 23)	80.00% (8 of 10)
<b>Makria</b>	13500	0.5	5	3	87.50% (14 of 16)	92.31% (12 of 13)
<b>Mando</b>	4	0.3	2	4	100% (15 of 15)	100% (11 of 11)
<b>Megalo Fteno</b>	9580	0.06	20	0	100% (13 of 13)	92.86% (13 of 14)
<b>Mikro Fteno</b>	5000	0.03	4	0	100% (10 of 10)	90.91% (20 of 22)
<b>Pachiea</b>	11850	1.36	7	1	83.33% (10 of 12)	72.73% (8 of 11)
<b>Tinos</b>	5800	194.5	1	6	47.06% (8 of 17)	41.67% (5 of 12)

**Table 1A: Island characteristics (Poulos *et al.*, 2009 & Semegen, 2018)**

<b>Island</b>	<b>Male Parasitemia</b>	<b>Female Parasitemia</b>	<b>Male BMI</b>	<b>Female BMI</b>	<b>Male Sprint</b>	<b>Female Sprint</b>	<b>Male Stamina</b>	<b>Female Stamina</b>
<b>Amorgos</b>	24.04	1	-0.144	-0.139	1.84	1.74	78.54	84.00
<b>Anafi</b>	92.14	25.169	0.097	0.324	1.74	1.77	127.00	94.90
<b>Andros</b>	163.267	69.63	-1.041	-0.451	1.87	2.07	55.00	48.50
<b>Ano Antikeros</b>	1.98	2.01	0.348	0.343	1.56	1.69	116.11	124.89
<b>Fidoussa</b>	37.257	8.07	-0.111	-0.509	1.73	1.86	63.46	74.71
<b>Glaronissi</b>	16.849	25.665	0.084	-0.126	2.05	1.92	117.67	100.29
<b>Ios</b>	7.44	NA	-0.346	-0.510	1.82	1.76	78.91	87.43
<b>Iraklia</b>	4.94	23.815	0.407	-0.332	1.60	1.82	108.20	68.14
<b>Keros</b>	24.165	27.383	0.266	-0.008	1.52	1.65	171.70	286.00
<b>Kisiri</b>	279.66	257.594	-0.521	-0.703	1.55	1.50	54.44	53.56
<b>Lazaros</b>	4.327	4.82	0.996	1.662	1.38	1.40	260.22	192.78
<b>Makria</b>	46.831	153.925	-0.157	0.156	1.71	1.65	126.71	133.22
<b>Mando</b>	42.929	64.643	-0.162	-0.526	2.73	2.64	59.71	55.00
<b>Megalo Fteno</b>	23.787	39.235	-0.480	-0.157	1.41	1.41	73.43	75.00
<b>Mikro Fteno</b>	13.434	58.997	-0.278	-0.168	1.75	1.57	244.67	128.50
<b>Pachia</b>	15.819	23.141	0.631	0.673	1.97	1.76	86.17	139.00
<b>Tinos</b>	47.65	30.716	-0.167	-0.019	2.11	1.97	50.44	48.30

Table 1B: Island characteristics – these were calculated from averaging value across islands or sex

	b1	t-value	P-value
Parasitemia (Ln transformed)	-0.144001	-3.562	<b>0.0005</b>
Hindspan	-0.003583	-0.394	0.695

**Table 2: Summary table of stamina regressed with parasitemia and hindspan as covariates. This output suggests that other factors, such as lizard hindspan does not affect a lizard's stamina.**

<b>Blood characteristics</b>	<b>Sample size (n)</b>	<b>Infected mean of cells +/- standard error</b>	<b>Uninfected mean of cells +/- standard error</b>	<b>Relative % Change</b>
<b>Basophils</b>	318	2.705 +/- 0.214	3.583 +/- 0.345	27.93%
<b>Eosinophils</b>	442	10.476 +/- 0.510	10.604 +/- 0.728	1.214%
<b>Heterophils</b>	456	21.096 +/- 0.805	25.368 +/- 1.334	18.39%
<b>Lymphocytes</b>	458	23.82 +/- 1.034	30.890 +/- 2.227	25.84%
<b>Monocytes</b>	444	8.045 +/- 0.372	11.463 +/- 0.937	35.042%
<b>Thrombocytes</b>	444	64.855 +/- 3.175	63.964 +/- 5.258	1.38%

**Table 3: Summary table of infection status and blood characteristic concentrations**

<b>Model</b>	<b>AIC</b>	<b>BIC</b>	<b>logLik</b>	<b>Deviance</b>	<b>Chi-sq</b>	<b>P</b>
Null	1126.5	1155.4	-556.25	1112.5		
Full	1064.8	1097.8	-524.40	1048.8	63.7	<b>1.449e-15</b>

**Table 4: Statistics summary of first likelihood ratio test between null and full model for BMI with island as focus factor. By including island factors, we increase the accuracy of our model.**

<b>Model</b>	<b>AIC</b>	<b>BIC</b>	<b>logLik</b>	<b>Deviance</b>	<b>Chi-sq</b>	<b>P</b>
Null	3512.9	3535.0	-1750.4	3500.9		
Full	3466.6	3492.4	-1726.3	3452.6	48.234	<b>3.784e-12</b>

**Table 5: Statistics summary of likelihood ratio test between null and full model for Max Stamina with island as focus factor. By including island factors, we increase the accuracy of our model.**

<b>Model</b>	<b>AIC</b>	<b>BIC</b>	<b>logLik</b>	<b>Deviance</b>	<b>Chi-sq</b>	<b>P</b>
Null	502.77	527.55	-245.39	490.77		
Full	287.30	316.20	-136.65	273.30	217.48	<b>2.2e-16</b>

**Table 6: Statistics summary of likelihood ratio test between null and full model for Max Stamina with island as focus factor. By including island factors, we increase the accuracy of our model.**

<b>Model</b>	<b>AIC</b>	<b>BIC</b>	<b>logLik</b>	<b>Deviance</b>	<b>Chi-sq</b>	<b>P</b>
Random Intercept	1112.6	1137.4	-550.29	1100.6		
Random Slope	1111.7	1144.7	-547.83	1095.7	4.905	0.086

**Table 7: Statistics summary of first likelihood ratio test between null and full model for BMI with island as focus factor. By including island factors, we increase the accuracy of our model.**

<b>Model</b>	<b>AIC</b>	<b>BIC</b>	<b>logLik</b>	<b>Deviance</b>	<b>Chi-sq</b>	<b>P</b>
Random Intercept	3468.5	3490.6	-1728.2	3456.5		
Random Slope	3472.5	3501.9	-1728.2	3456.5	0.003	0.9985

**Table 8: Statistics summary of likelihood ratio test between null and full model for Max Stamina with island as focus factor. By including island factors, we increase the accuracy of our model.**

<b>Model</b>	<b>AIC</b>	<b>BIC</b>	<b>logLik</b>	<b>Deviance</b>	<b>Chi-sq</b>	<b>P</b>
Random Intercept	285.61	310.39	-136.81	271.61		
Random Intercept	287.71	320.75	-135.86	271.71	1.899	0.387

**Table 9: Statistics summary of likelihood ratio test between null and full model for sprinting with island as focus factor. By including island factors, we increase the accuracy of our model.**

<b>Island Variable</b>	<b>BMI</b>	<b>Max Stamina</b>	<b>Max Sprint Trial</b>
<b>Lizard Density</b>	<b>P=0.019</b>	P= 0.255	NA (Redundant variables)
<b>Number of Predators</b>	NA (Redundant variables)	P= 0.78	<b>P= 0.030</b>
<b>Island Age</b>	P= 0.850	P= 0.236	P= 0.119

**Table 10: P-value summary of likelihood ratio tests between the mixed models, with or without specific island characteristics. The inclusion of more specific island variables, such as density or predators improve models for BMI and sprinting (respectively). NA outputs suggest a high correlation between island identity and the characteristic examined.**

## Appendix

$$Y = \text{LnParasitemia} + \text{Sex} + \text{Infection Status}$$

$$Y = \text{LnParasitemia} + \text{Sex} + \text{Infection Status} + (1|\text{Island})$$

**Equation 1: Initial null model and full model for comparative analysis, where Y represents BMI, stamina, or sprint trials**

$$Y = \text{LnParasitemia} + \text{Sex} + \text{Infection Status} + (1|\text{Island})$$

$$Y = \text{LnParasitemia} + \text{Sex} + \text{Infection Status} + (1 + \text{LnParasitemia}|\text{Island})$$

**Equation 2: Random intercept and random slope mixed models for comparative analysis, where Y represents BMI, stamina, or sprint trials. The**

$$Y = \text{LnParasitemia} + \text{Sex} + \text{Infection Status} + (1|\text{Island})$$

$$Y = \text{LnParasitemia} + \text{Sex} + (1|\text{Island}) + (1|F)$$

**Equation 3: Subsequent null model vs full model for mixed model analysis, where Y represents BMI, stamina, or sprint trials and F represents the focus factor (Island Area, Age, Lizard Density, Number of Predators)**

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