# *EFFECT OF POPULATION FRAGMENTATION ON HOST-PARASITE INTERACTIONS: INSIGHTS FROM AN ISLAND LIZARD*

Johanna Fornberg

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**ABSTRACT**

I assessed the effect of island characteristics and population isolation on the endemic insular lizard *Podarcis erhardii* and its native hemogregarine parasite *Hepatozoon spp.* I analyzed the relationships of prevalence, infection likelihood, and parasitemia to several factors at the island (time of isolation, area, distance to nearest larger land mass), population (host density), and organismal (load of hematophagous ectoparasites) levels. My results suggest that smaller islands, as well as islands that have been isolated for longer periods of time, show higher infection rates and higher parasitemia in hosts than others. I also found that distance between a focal island to the nearest larger land mass, as well as the load of hematophagous ectoparasites on an individual, were poor predictors of infection variables in *P. erhardii*. These results indicate that island area, host population density, and island age are likely to be significant drivers of changes in host-parasite interactions in fragmented populations.

**KEYWORDS**

Host-parasite interactions, hemogregarines, lizards, biogeography

## INTRODUCTION

Host-parasite interactions show complex dynamics, which shape and are shaped by the surrounding ecosystem (Hess 1996, McCallum and Dobson 2002). Spatial heterogeneities, such as fragmentation and isolation of habitat patches in particular, can affect transmission dynamics of many pathogens and parasites (Perkins 2001, Rees et al. 2013). The ecology of host populations can also be affected by biogeography, which can shape the distribution of parasites in an ecosystem. Islands may effectively isolate hosts and parasites; they also have the potential to modify both parasite transmission and its ultimate impact on host populations (Roca et al. 2009, Pérez-Rodríguez et al. 2013, Foufopoulos et al. 2017). More specifically, island biogeography can influence host-parasite interactions through direct changes in transmission, such as changing vector distributions (Perkins 2001, Clark and Clegg 2014), or by altering genetic diversity (Plaisance et al. 2008, Roca et al. 2009, Koop et al. 2014) of hosts and parasites.

Studies on island biogeography have shown that the size of an island, distance between a focal island and a larger "source" land mass facilitating overwater dispersion, and the amount of time an island has been isolated from the mainland can dramatically change the ecology of host and parasite populations (Dobson et al. 1992, Galdón et al. 2006, Roca and Galdón 2009, Foufopoulos et al. 2017). Island biogeography can shape the behavior, physiology, disease susceptibility, and genetic diversity of insular populations and communities (Schall 1996, Foufopoulos and Ives 1999, Hurston et al. 2009, Slavenko et al. 2014). For example, Hurston et al. (2009) found that genetic diversity of native reptile populations in the Aegean islands was significantly reduced as islands were isolated for longer periods.

An isolated habitat also leads to changes in population density and species interactions, notwithstanding host-parasite dynamics (Dobson et al. 1992, Roca et al. 2009). Garrido & Pérez-Mellado (2013) found that island hosts had significantly higher parasite loads and greater prevalence of parasitism as a result of reduced genetic variability and increased host population

density. Concomitant with traditional island biography theory (MacArthur and Wilson 1967), island effects on host-parasite interactions are most apparent in relation to changes in population density and island area – two factors which are often naturally interrelated in island systems (MacArthur and Wilson 1967, Dobson et al. 1992, Galdón et al. 2006).

Studies investigating the relationships between parasite infection and island characteristics have demonstrated that isolation tends to lead larger islands (and, especially, mainland and 'source' islands), and denser host populations, to harbor higher parasite prevalence, as well as more substantial parasite burdens (Gouy de Bellocq et al. 2002, Roca et al. 2009). Foufopoulos et al. (2017) found, for example, that denser host populations had significantly larger parasite loads of gut parasites in Aegean reptile populations. Similar research also suggests that longer periods of isolation lead to a distinct loss of genetic diversity in the parasite and subsequently impoverished parasite communities in host populations (Roca et al. 2009, Pérez-Rodríguez et al. 2013, Koop et al. 2014). Such is the case, for example, of the Roca et al. (2009) study which found helminths communities to be increasingly depauperate the longer the period of isolation.

I assessed the host-parasite interactions between an insular lizard species (*Podarcis erhardii,* Reptilia: Lacertidae*)* and its native vector-transmitted hemogregarine parasite (*Hepatozoon complex,* Apicomplexa: Adeleorina*)*, in a continental land-bridge island system (Cyclades, Aegean Sea, Greece). The Cycladic islands are an archipelago of land-bridge islands located off the eastern coast of Greece (Figure 2). Most of the islands, and the vertebrate populations inhabiting them, became progressively isolated during the gradual rise in sea levels following the last glacial maximum 18000 years ago (Pirazolli 1991, Poulos et al. 2009, Kapsimalis et al. 2009). Duration of island isolation depends on the depth of the underwater saddle between two islands and varies greatly between 5 years and 5.33 million years (Foufopoulos and Ives 1999). Multiple lines of evidence indicate that *P. erhardii* is a very poor over-water disperser; as a result, the evolutionary history of Cycladic populations reflects

the fragmentation history of the islands they inhabit (Foufopoulos and Ives 1999, Hurston et al. 2009). Because of the resulting diversity of population characteristics, the Cycladic islands present an excellent study system to investigate the long-term effects of habitat fragmentation on vertebrate populations in general and the evolutionary ecology of host-parasite interactions in specific.

I determined how island characteristics and host ecology relate to the prevalence, infection likelihood, and parasite loads ("parasitemia") of *Hepatozoon* in populations of a native reptile, *Podarcis erhardii*. Consistent with island biogeography theory, I hypothesized that island characteristics would significantly influence the prevalence and parasitemia of *Hepatozoon spp.* infections in *P. erhardii* populations. Specifically, I predicted that prevalence and parasitemia would increase with increasing island size and decreasing island age, as well as on islands which are closer to larger land masses (cf. MacArthur and Wilson 1967, Roca et al. 2009). I also predicted that denser host populations, and greater prevalence of hematophagous ectoparasites (*Ophionyssus sp.* mites, a suspected invertebrate host), would be positively correlated with *Hepatozoon spp.* infection and parasitemia in *P. erhardii*.

## METHODS

### *Study System*

The Cycladic island archipelago is comprised of several hundred islands and islets. Larger islands are covered by a patchwork of open habitats, ranging from sclerophyllous maquis and coastal heaths (termed 'phrygana') to agricultural areas, riverine thickets, and exposed rock glades. The islands experience typical Mediterranean climate with wet, mild winters and hot, dry summers. In total, I sampled 19 Cycladic islands in this study. Islands sampled were chosen to represent a sufficiently broad range of periods of isolation (4 to 200000 years) and sizes (0.008 to 379.95 $km^2$) to reflect the varying conditions in the island system (Table 1). Sampling was conducted at low elevation (< 500 m above sea level) sites dominated by phrygana or macchia shrubland.

*Podarcis erhardii* (Reptilia: Lacertidae), commonly known as the Erhard's wall lizard or Aegean wall lizard, is a ground-dwelling lizard endemic to the southern Balkans. *P. erhardii* ranges as far north as Bulgaria and west into Albania, and is found throughout mainland Greece and the Aegean islands, including the Cyclades archipelago (Valakos et al. 1999). *P. erhardii* is a medium-sized lizard, with snout-vent length typically between 49-78 mm. It is a generalist insectivore occurring across most of the habitat types in the region (Figure 1) (Valakos et al. 2008). The complex geologic history of the Cycladic islands have driven notable divergence in *Podarcis* morphology (Donihue 2016, Brock et al. 2015), behavior (Pérez-Mellado et al. 1997, Donihue et al. 2015, Deem and Hedman 2014), and genetic diversity (Poulakakis et al. 2003, Hurston et al. 2009). *Podarcis* also shows variation in parasite loads (Roca et al. 2009, Foufopoulos et al. 2017) of *P. erhardii* populations. In natural habitats, *P. erhardii* is commonly infected with mites (*Ophionyssus sp.*, Acari: Trombiculidae) and with ticks (*Haemaphysalis* sp., Acari: Ixodoidea) on islands with domestic livestock (Pafilis et al. 2013). *Podarcis* lizards are naturally infected with hemogregarine parasites (Garrido and Pérez-Mellado 2013); this includes infection by *Hepatozoon spp.* (James Harris et al. 2012, Damas-Moreira et al. 2014), in native populations.

*Hepatozoon* parasites (Apicomplexa: Adeleorina) are a genus of hemogregarine parasites that infect red blood cells (RBCs) of their hosts. *Hepatozoon spp.* infect a wide range of vertebrate species, and occur across all orders of Reptilia (Telford 2008). *Hepatozoon* is also found in diverse groups of invertebrates, including mites (Acari), fleas (Siphonaptera), sandflies (Phlebotominae), and others. *Hepatozoon* parasites have a complex life cycle that is highly variable across species, often adapted to the host's biology (Smith 1996). In general, the reproductive cycle of *Hepatozoon* parasites require a definitive invertebrate host, and intermediate vertebrate host (Smith 1996, Roca and Galdón 2009). Transmission between invertebrate and vertebrate hosts typically occurs via consumption of an infected invertebrate or vertebrate by an intermediate vertebrate host (Smith 1996). The effects of *Hepatozoon* infection

on reptile hosts, including *Podarcis* lizards, are not widely understood. Several studies have found *Hepatozoon* infection to be largely asymptomatic (Caudell et al. 2002, Telford 2008). Others, however, have found *Hepatozoon* to affect host condition (Garrido and Perez-Mellado 2012) or immunosuppression and anemia (Telford 1984) in free-ranging reptile hosts.

*Data Collection*

Data on several potential causative factors influencing host-parasite ecology in this system were collected. Island age was calculated using bathymetric data and historic estimates of rising sea levels in the region during the Pleistocene until the present (see Brock et al. 2015). Island area is represented as the size of islands in square kilometers. Host population density was determined as the number of individuals seen across a 100-meter transect (indiv./100-m) on each island at the site of sampling. Distance was determined as the distance (in kilometers) separating the focal island to the nearest larger land mass (*i.e.* the potential source land mass for overwater dispersers).

I sampled the resident *P. erhardii* populations at a single representative site on each island (see: Figure 2). I collected between 18 and 35 individuals from each island, using hand-held nooses. All collections were conducted in May to July of 2016, during the main reproductive period of *P. erhardii*. Capture coincided with favorable climatic conditions: sunny, wind less than 3 on the Beaufort scale, and temperature between 20-25$^{o}$C. Lizard capture was also coincident with peak activity periods for the species: early morning and late afternoon.

Once lizards were caught, body mass, snout-vent length, sex, and tail condition (autotomized vs. intact) were recorded for each individual. To assess the relationship between *Hepatozoon spp.* and *P. erhardii* more thoroughly, I accounted for hematophagous mite (*Ophionyssus sp.*) loads on lizards sampled, to approximate their role as vectors of *Hepatozoon spp.* Blood samples were taken from each lizard via toe-clipping (see Schall 1990, Langkilde et al. 2006). Blood smears were prepared on microscope slides and dried at ambient temperatures, then fixed using methanol. Blood smears were subsequently stained using a

hematoxylin and eosin stain (Fisher HealthCare™ Hema 3™ Manual Staining System). I then released each lizard at the site of capture.

Blood parasites were identified visually based on morphological characteristics, as per Telford (2008) (Figure 3). I determined presence of *Hepatozoon spp.* and parasitemia in infected hosts by scoring blood smears for the presence of infected mature erythrocytes using optical microscopy at 1000x magnification in oil immersion. Each smear was examined until 10000 mature red blood cells had been evaluated. Prevalence was calculated as percent of infected individuals out of total sampled from each island population. Infection likelihood was assessed as the probability of an individual being infected (carrying 1 or more parasites) or uninfected (carrying no parasites). Parasitemia per individual was calculated as the number of infected red blood cells out of 10000 red blood cells; average parasitemia was calculated as the average parasitemia per individual for all infected lizards in an island sample.

*Statistical Analyses*

All statistical analyses were conducted in RStudio (version 1.0.136, J.J. Allaire). Infection variables (prevalence, infection likelihood, and parasitemia) were analyzed against various factors (island area, island age, host density, distance, and ectoparasite loads) at several scales. Relationships between prevalence and island factors were assessed using simple and multiple regression models. Because of the substantial number of non-infected animals in some populations, I analyzed infection likelihood and parasitemia of individual hosts using a zero-inflated negative binomial regression model. Zero-inflated negative binomial regression models allowed me to account for zero-inflation in the data, which originates from these uninfected hosts, by assessing the infection likelihood of each individual, i.e. the probability that an individual will be infected or uninfected. This model also accounted for over-dispersion of parasitemia across infected individuals (Greene 2008). Island-, population-, as well as individual-level characteristics were included as predictors of parasitemia in all relevant zero-inflated negative binomial models.

## RESULTS

I found that *Hepatozoon spp.* was ubiquitous across all 19 study islands, but its prevalence varied widely. Infection prevalence ranged between 7% in the island population on Antikeros to 100% on the island Mando; on average, 62% of all lizards were infected with *Hepatozoon spp.* (511 total individuals sampled: Table 1). I detected no difference in prevalence between sexes based on a Welch's two-tailed t-test ($N_{female}$ = 208, $N_{male}$ = 303, p = 0.34). Overall, parasite loads in infected individuals were light (<100 infected RBCs per 10000 RBCs) and parasitemia of infected individuals ranged from 1 to 1211 infected RBCs per 10000 RBCs. The average parasitemia for all infected *P. erhardii* individuals was 43 (+/- 10.59 SE) infected RBCs out of 10000 RBCs; average parasitemia for *P. erhardii* populations ranged from 2 to 270 infected RBCs out of 10000 RBCs (Table 1). There were also no detectable differences in parasitemia between sexes based on a Welch's two-tailed t-test ($N_{female}$ = 208, $N_{male}$ = 303, p = 0.93).

*Models of Prevalence*

I used several predictors to assess the relationship between prevalence of *Hepatozoon* spp. infection and the ecology and natural history of each insular population. At the island level, I tested the relationship of prevalence with island area ($km^2$), time of isolation of each island population (years), and the distance of each island to the nearest larger 'source' land mass (km). I investigated host density (no. of individuals detected along a 100-meter transect) on each island as a population-level variable.

In single variable regression models, host density was marginally significant predictor of infection prevalence (β = 0.16, adjusted $R^2$ = 0.16, p = 0.051). The relationship between prevalence and island age was also marginally significant (β = -0.06, adjusted $R^2$ = 0.16, p = 0.053). I detected a marginally non-significant negative trend between island area and *Hepatozoon* prevalence (β = -0.05, adjusted $R^2$ = 0.12, p = 0.083). There was no significant relationship between infection prevalence and distance to source island (β = -0.10, adjusted $R^2$

= 0.08, p = 0.121; Table 2).

I combined predictors in multiple regression models, and compared their performance based on calculated Akaike information criterion (AIC) values (Burnham and Anderson 2002). The best model included island age as well as host density (adjusted $R^2$ = 0.413, p = 0.006, AIC = 0.743; Figure 4). It suggested that *Hepatozoon* prevalence increased on islands supporting denser lizard populations (β = 0.18, p = 0.010) and declined in those island populations that have been isolated for longer periods of time (β = -0.08, p = 0.011). The second-best model included island area in addition to host density and island age, (adjusted $R^2$ = 0.39, p = 0.016, AIC = 2.39), indicating that prevalence increased on smaller islands; this may in part be explained by the increased lizard densities (and presumably elevated transmission rates on smaller islands: Table 2).

*Models of Parasitemia*

I used zero-inflated negative binomial regression models to analyze the relationship between infection likelihood (*i.e.*, the probability of an individual being infected) and parasitemia (the number of infected red blood cells out of 10000 RBCs), and several island, population, and organismal-level predictors. I considered several island level variables: island area ($km^2$), time of isolation of each island (years), the distance to a source island (km). Host density was included as a population level variable. The load of ectoparasitic mites on each host was included as an individual level variables.

I determined the best-fitting model using AIC values (Burnham and Anderson 2002). The best model included island age, island area, host density, and the interaction term between host density and island area (Table 3). These three variables were the strongest predictors of parasitemia, as well as infection likelihood in individual hosts in all model simulations. For the parasitemia variable in these models, island age (β = -0.97, p < 0.001) and island area (β = -0.21, p = 0.006) demonstrated significant negative relationships with parasitemia in individual hosts. Host density yielded a significant positive relationship with parasitemia (β = 0.10, p =

0.043). I found that distance to source and mite loads were not significant predictors in these model iterations.

Assessing the zero-inflation component of this model, I found that island age ($p < 0.001$) and island area ($p = 0.189$) were negatively associated with infection likelihood of individuals; however, host density was positively associated with infection status. I also found that distance to a source land mass was not a significant predictor of the infection status of individuals. These trends parallel results of prevalence of *Hepatozoon spp.* infection at the population level. Taken together, these results suggest that the time of isolation of an island as well as the island area and the density of host populations are important determinants of the prevalence and parasitemia of *Hepatozoon* parasites infecting *P. erhardii*. This suggests that such factors could be important predictors for the distribution and intensity of parasitism in insular or fragmented populations.

**DISCUSSION**

The effects of fragmentation and isolation of landscapes on the ecology of host-parasite interactions are complex and often mediated through a variety of ecological processes. The natural history of land-bridge islands, and particularly the complex geologic processes that form them, reflect the natural history of insular populations and can ultimately inform their evolutionary responses to isolation (Foufopoulos and Ives 1999, Hurston et al. 2009). The results presented here support the idea that island characteristics can shape the ecology of insular host populations, and, subsequently, the interactions between parasites and hosts.

I show that for host-parasite interactions, changes in spatial structure and population ecology can significantly alter infection rates and parasite loads found in hosts by two primary mechanisms. The first mechanism concerns changes in host population density initiated, in part, by changing island size; the second is changes in genetic variability as a result of longer time of isolation.

Specifically, my results indicate that increasing island area is a significant island

characteristic that reduces both prevalence and likelihood of *Hepatozoon spp.* infection, as well as parasitemia of infections in the host *P. erhardii* in Aegean island populations. The size of an island was negatively related to prevalence, infection likelihood, and parasitemia in *P. erhardii* populations. As a result, smaller islands harbor more infected individuals, as well as greater parasite loads in individuals. Two of the smallest islands sampled, Kisiri and Mando, also had especially high prevalence (96% and 100%, respectively) and average parasitemia (270 and 53 infected RBCs per 10000 RBCs, respectively) of all islands sampled.

I also found significant positive relationships between host density and prevalence, infection likelihood, and parasitemia. Overall, I found that lizard populations are significantly denser on smaller Cycladic islands. The resulting associations between *Hepatozoon spp.* infection with island area and host density, taken together, support ecological expectations adherent to the mechanism of density compensation (Wright 1979), where host density is greater on smaller islands and infection is subsequently more prevalent and intense.

Prevalence, infection likelihood, and parasitemia are greater in smaller, denser insular populations; this indicates that population ecology and abiotic spatial structure shape the distribution and intensity of *Hepatozoon* parasitism in this system. For example, on the small island Megalo Fteno (area: 0.06 $km^2$, host density: 20 indiv./100-m) prevalence (96%) and average parasitemia (32.5 infected RBCs per 10000 RBCs) were particularly higher than on the larger, less dense island Antikeros (area: 1.05 $km^2$, host density: 1 indiv./100-m) where prevalence was only 7%, and average parasitemia was 1.99 infected RBCs per 10000 RBCs.

I found this pattern in *P. erhardii* populations: denser populations demonstrate greater prevalence, infection likelihood, and parasitemia. More interestingly, I also see that effects of island area emphasize this increase in infection: islands that are smaller also show an increase in infection and parasite load. The significant interaction between island area and host density in these models supports that the impact of island area and host population density are interconnected, and drive changes in host-parasite ecology in this system by increasing

infection and parasitemia in smaller, denser *P. erhardii* populations. These results support the occurrence of density compensation, whereby increased population density of hosts coupled with smaller habitat size leads to increased rates of parasitism (Keesing et al. 2006, Keesing et al. 2010).

The time since isolation of each island was negatively related to prevalence, infection likelihood, and parasitemia. Island populations that have been isolated for increasingly long periods of time have lower prevalence of *Hepatozoon* and lower levels of parasitemia. One explanation could be that reduced genetic diversity of isolated populations of *Hepatozoon* leads to impoverished parasite populations in insular communities isolated for long periods. Genetic diversity is reduced over time following isolation of a population, and the implications of reduced genetic variability in hosts as well as in parasites has cascading impacts on host-parasite interactions (Plaisance et al. 2008, Hurston et al. 2009). This study shows that these impacts crystallize as lower abundance of parasites in more isolated host populations, as well as lower parasitemia, as time of isolation for an island increases. This outcome mirrors the results of a similar study (Roca et al. 2009) on *P. erhardii* and helminth communities. My results show that older islands have lower *Hepatozoon* prevalence and infection intensity in *P. erhardii* hosts. This pattern suggests that loss of genetic diversity over evolutionary time reduces the ability of parasites to maintain transmission cycles and results in a greater parasite prevalence in hosts (Roca et al. 2009).

These results also show, perhaps counterintuitively, that distance to a larger source island is not significant in predicting infection rates or parasitemia for *P. erhardii* and *Hepatozoon spp.* infections. The absence of a distance effect is best interpreted as a lack of overwater transmission of either hosts or pathogens. This observation is in line with other studies in the region, indicating the lack of overwater dispersal for *Podarcis* over ecological time (Wettstein 1953, Foufopoulos and Ives 1999, Hurston et al. 2009). Possibly, *P. erhardii* does not therefore incur changes in transmission through transport of infectious agents between islands,

such as by dispersal of invertebrate hosts, due to this lack of dispersion between islands.

Similarly, mites were also not significantly related to infection or parasitemia in *P. erhardii*. While I theorized that mites in this system might be a definitive infectious host of *Hepatozoon spp.*, these results suggest that they may not be significant transmitters of these hemoparasites to *P. erhardii*. However, there may be a more complex underlying driver of invertebrate infectious agents in this island system, mediated by island effects which could not be properly accounted for in this study. For example, there may be other island characteristics or biotic processes that affect the interactions between mites with *Hepatozoon spp.* and *P. erhardii*, such as nutrient availability or predation regimes. Mites may also not be an infectious host for *Hepatozoon spp*. Further investigations into the transmission biology of *Hepatozoon spp.*, would therefore be an unexplored and valuable subject for further research.

The significance of island area, host density, and island age on the distribution and intensity of *Hepatozoon spp.* infections in *P. erhardii* populations demonstrate several important ecological changes that occur following isolation of insular populations. The loss of predation pressure and interspecies competition on islands, and concomitant changes in prey population density, lead to notable changes in the ecology of insular species (Blumstein and Daniel 2005, Keesing et al. 2006, Li et al. 2014). The influence these changes have on host-parasite interactions has previously received less attention in disease ecology literature or in studies on the biogeography of parasites.

This study provides a model of how host-parasite interactions can change over evolutionary time when populations become isolated following fragmentation. I found that changes in host population characteristics through shifts in the amount of available habitat and density of populations, can effectively change the prevalence and intensity of infection. These results also suggest that reduction in genetic diversity (a function of how long a population has been isolated) reduces parasitemia and infection rates. This suggests that island effects at the population level, as well as at the genetic scale, are important in driving changes in host-

parasite dynamics. In particular, the size of island habitats and the density of populations on those islands significantly alter the interactions between hosts and *Hepatozoon spp.* parasites through the mechanism of density compensation following isolation (Wright 1979, Keesing et al. 2006). The time for which islands are isolated also affects these interactions over evolutionary time by impacting the genetic variability of hosts and parasites. The influence of these island characteristics on host-parasite interactions implies that parasitism can be significantly altered based on the characteristics of island or island-like fragments. Such implications of important for when predicting how fragmentation may alter host-parasite ecology in natural systems.

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## FIGURES

***Figure 1.*** *Aegean Wall lizard (Podarcis erhardii) on the Aegean Island of Naxos. Inset: red blood cells infected with Hepatozoon spp. in P. erhardii.*

***Figure 2.*** *Map of islands sampled. Islands in the Cyclades were chosen based on variety of age (in years) and area (in km$^2$) of each island. Islands sampled are shown in grey. Island acronyms: Amorgos (AM), Anafi (AF), Andros (AN), Antikeros (AT), Fidoussa (FD), Glaronissi (GL), Ios (IO), Iraklia (IR), Keros (KE), Kisiri (KS), Lazaros (LZ), Makria (MK), Mando (MN), Megalo Fteno (MG), Mikro Fteno (MF), Naxos (NX), Pacheia (PC), Parthenos (PR), Tinos (TN).*

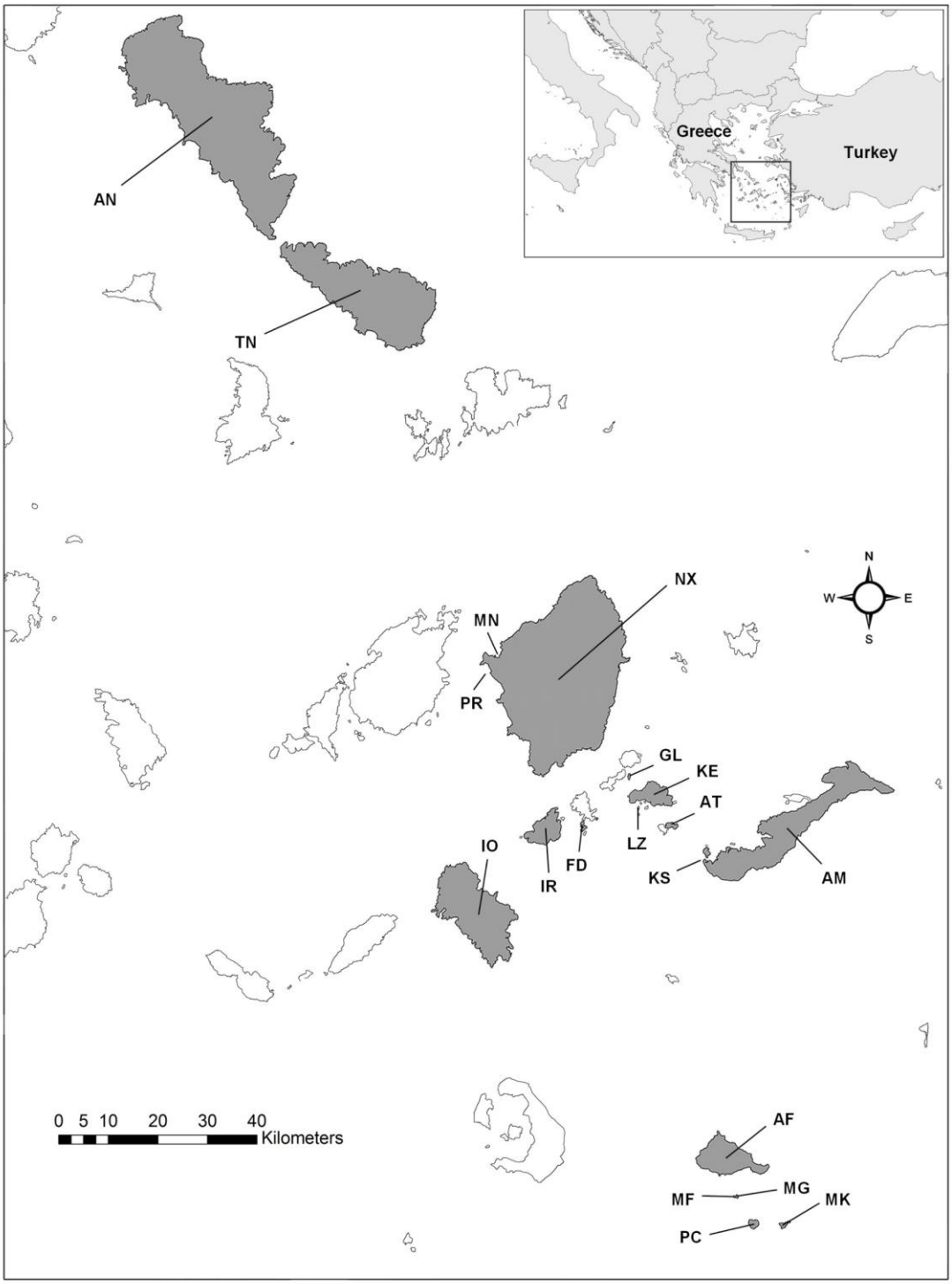


***Figure 3.*** *Infected mature erythrocytes from a blood smear of P. erhardii. Hepatozoon spp. gametocytes can be seen within the cell membrane of P. erhardii erythrocytes (black circles).*

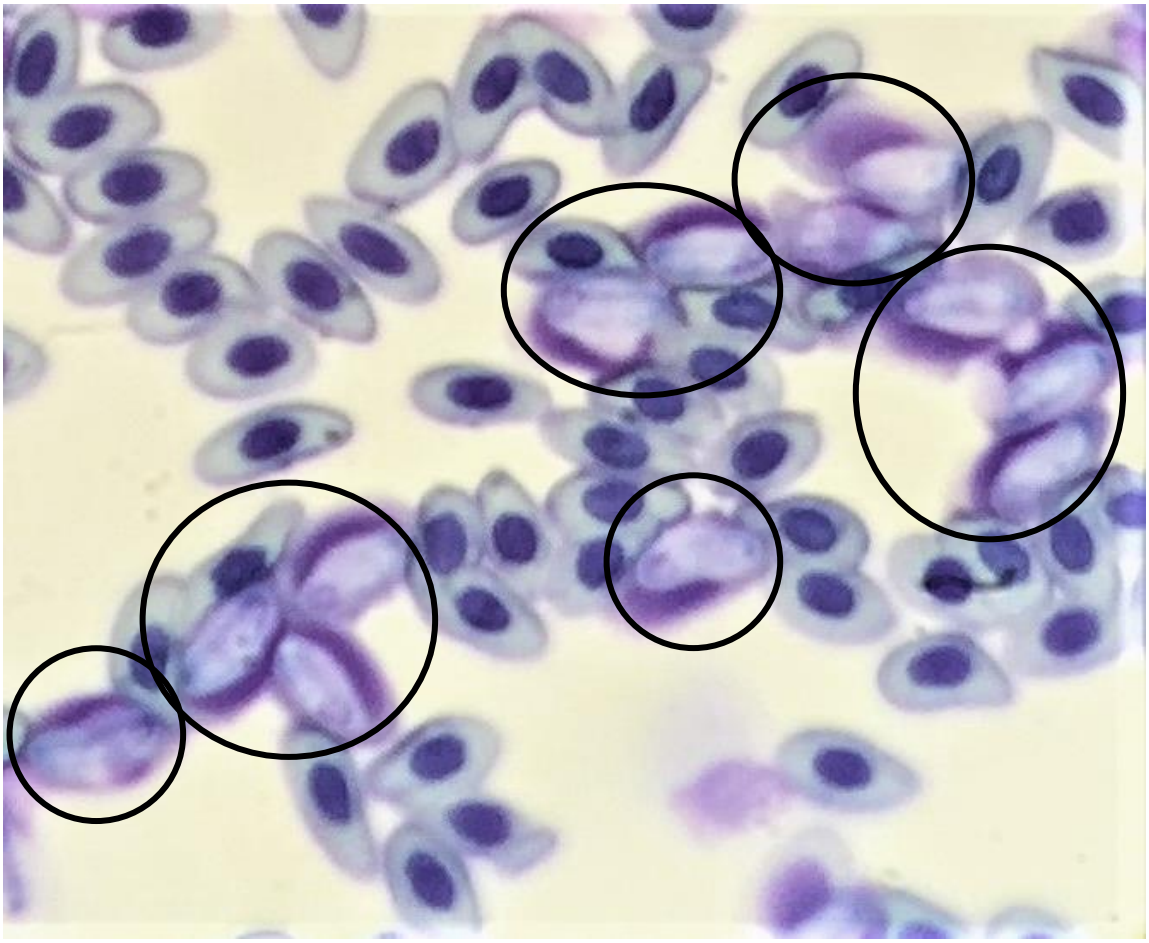
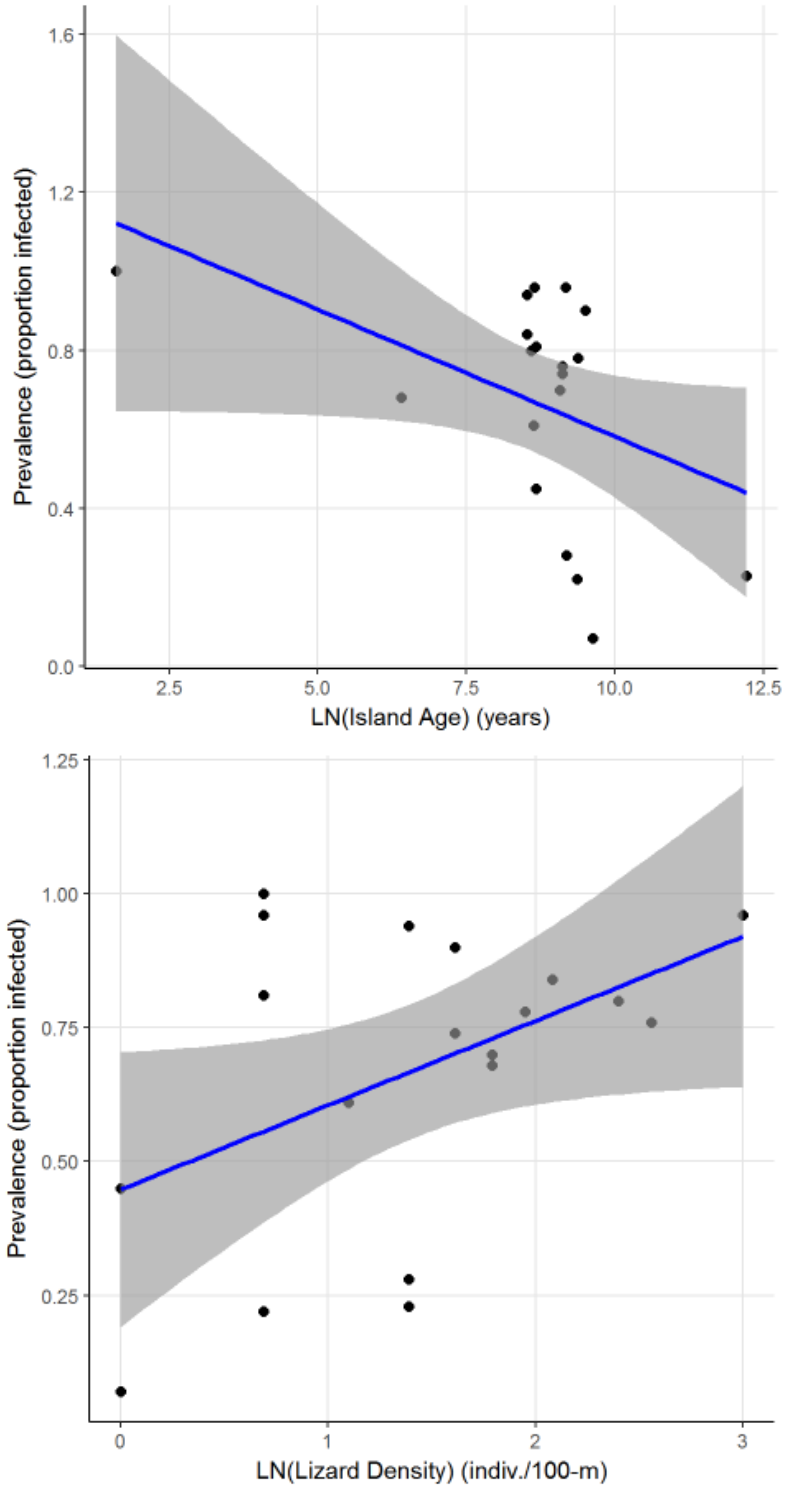


***Figure 4.*** *Relationship between prevalence (percent infected individuals) and island age (in years) and lizard density (no. of individuals detected along a 100-meter transect).*



## TABLES

***Table 1.*** *Infection and island characteristics of P. erhardii populations infected with Hepatozoon spp. in sampled island populations.*

| Island | N | *Hepatozoon spp.* Prevalence (%) | Average Parasitemia | Island Age (years) | Island Area ($km^2$) | Host Density (indiv./ 100-m) | Distance (km) |
|---|---|---|---|---|---|---|---|
| Amorgos | 22 | 23 | 19.43 | 200000 | 123 | 4 | 25.117 |
| Anafi | 32 | 84 | 69.82 | 5000 | 40.37 | 8 | 20.85 |
| Andros | 21 | 81 | 146.61 | 5800 | 379.95 | 2 | 11.98 |
| Antikeros | 30 | 7 | 1.99 | 15150 | 1.05 | 1 | 6.885 |
| Fidoussa | 31 | 68 | 33.09 | 600 | 0.63 | 6 | 0.05 |
| Glaronissi | 23 | 61 | 19.37 | 5600 | 0.16 | 3 | 0.553 |
| Ios | 18 | 22 | 7.44 | 11750 | 109.03 | 2 | 18.1 |
| Iraklia | 25 | 28 | 10.33 | 9800 | 18.08 | 4 | 5.355 |
| Keros | 31 | 74 | 25.98 | 9150 | 15.05 | 5 | 8.925 |
| Kisiri | 28 | 96 | 269.86 | 5700 | 0.01 | 2 | 0.534 |
| Lazaros | 34 | 76 | 4.48 | 9100 | 0.01 | 13 | 1.108 |
| Makria | 29 | 90 | 96.26 | 13500 | 0.5 | 5 | 8.16 |
| Mando | 26 | 100 | 52.87 | 4 | 0.3 | 2 | 0.01 |
| Megalo Fteno | 27 | 96 | 32.5 | 9580 | 0.06 | 20 | 3.652 |
| Mikro Fteno | 32 | 94 | 43.81 | 5000 | 0.03 | 4 | 3.662 |
| Naxos | 30 | 70 | 64.29 | 8700 | 448 | 6 | 0 |
| Pacheia | 23 | 78 | 19.32 | 11850 | 1.36 | 7 | 8.41 |
| Parthenos | 20 | 80 | 15.82 | 5400 | 0.008 | 11 | 0.567 |
| Tinos | 29 | 45 | 41.14 | 5800 | 194.5 | 1 | 1.912 |

***Table 2.*** *Results of simple and multiple regressions on prevalence of Hepatozoon spp. infection*

| Model | Predictors | Estimate | SE | p | Adjusted $R^2$ | F | df | p | AIC |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Age | -0.06 | 0.03 | 0.053 | 0.16 | 4.32 | 1, 17 | 0.053 | 6.79 |
| 2 | Area | -0.05 | 0.03 | 0.083 | 0.12 | 3.40 | 1, 17 | 0.083 | 7.63 |
| 3 | Distance | -0.10 | 0.06 | 0.121 | 0.08 | 2.67 | 1, 17 | 0.121 | 8.32 |
| 4 | Host Density | 0.16 | 0.07 | 0.051 | 0.16 | 4.42 | 1, 17 | 0.051 | 6.70 |
| 5 | Age | -0.05 | 0.03 | 0.125 | 0.19 | 3.17 | 2, 16 | 0.069 | 6.75 |
| | Area | -0.04 | 0.03 | 0.197 | | | | | |
| 6 | Area | -0.04 | 0.03 | 0.198 | 0.20 | 3.22 | 2, 16 | 0.067 | 6.67 |
| | Host Density | 0.13 | 0.08 | 0.120 | | | | | |
| **7** | **Age** | **-0.08** | **0.03** | **0.011*** | **0.41** | **7.33** | **2, 16** | **0.006*** | **0.74** |
| | **Host Density** | **0.18** | **0.06** | **0.010*** | | | | | |
| 8 | Age | -0.07 | 0.03 | 0.028* | 0.39 | 4.76 | 3, 15 | 0.016* | 2.39 |
| | Area | -0.01 | 0.03 | 0.602 | | | | | |
| | Host Density | 0.17 | 0.07 | 0.027* | | | | | |
| 9 | Age | -0.07 | 0.03 | 0.034* | 0.34 | 3.35 | 4, 14 | 0.040* | 4.33 |
| | Area | -0.01 | 0.05 | 0.906 | | | | | |
| | Host Density | 0.18 | 0.09 | 0.059 | | | | | |
| | Area*Host Density | -0.01 | 0.03 | 0.840 | | | | | |

***Table 3.*** *Results of zero-inflated negative binomial models of infection status and parasitemia.*

| | | Count Model | | | Zero-Inflated Model | | | |
|---|---|---|---|---|---|---|---|---|
| **Model** | **Predictors** | **Estimate** | **SE** | **p** | **Estimate** | **SE** | **p** | **AIC** |
| 1 | Age | -0.02 | 0.05 | 0.662 | 1.09 | 0.21 | 2.87E-07* | 3942.7 |
| 2 | Area | -0.01 | 0.04 | 0.864 | 0.39 | 0.12 | 0.001* | 3961.9 |
| 3 | Distance | -0.11 | 0.09 | 0.216 | 1.77 | 1.67 | 0.286 | 3959.7 |
| 4 | Host Density | -0.67 | 0.12 | 3.52E-08* | -14.46 | 45.82 | 0.752 | 3896.0 |
| 5 | Mites | 0.11 | 0.01 | <2E-16* | 0.16 | 0.07 | 0.033* | 27819.5 |
| 6 | Age | 0.08 | 0.05 | 2.99E-11* | 1.36 | 0.27 | 5.94E-07* | 3844.8 |
| | Host Density | -0.79 | 0.12 | <2E-16* | -2.50 | 0.68 | 0.002* | |
| 7 | Area | 0.00 | 0.04 | 0.948 | 0.19 | 0.07 | 0.012* | 3905.1 |
| | Host Density | -0.72 | 0.11 | 2.17E-10* | -1.59 | 0.40 | 7.39E-05* | |
| 8 | Area | -0.20 | 0.08 | 0.008* | -0.49 | 0.19 | 0.009* | 3877.2 |
| | Host Density | -0.87 | 0.13 | 1.21E-11* | -7.90 | 2.25 | 0.000* | |
| | Area*Host Density | 0.14 | 0.05 | 0.008* | 1.23 | 0.36 | 0.001* | |
| 9 | Age | 0.08 | 0.05 | 0.110 | 1.32 | 0.28 | 1.98E-06* | 3848.5 |
| | Area | -0.01 | 0.04 | 0.808 | 0.05 | 0.09 | 0.590 | |
| | Host Density | -0.80 | 0.12 | 2.57E-11* | -2.39 | 0.69 | 0.001* | |
| **10** | **Area** | **-0.21** | **0.08** | **0.006*** | **-0.17** | **0.13** | **0.189** | **3839.5** |
| | **Age** | **-0.97** | **0.13** | **2.29E-14*** | **-3.14** | **0.88** | **3.68E-04*** | |
| | **Host Density** | **0.10** | **0.05** | **0.043*** | **1.01** | **0.21** | **2.39E-06*** | |
| | **Area*Host Density** | **0.15** | **0.05** | **0.003*** | **0.37** | **0.17** | **0.029*** | |