Original Contribution

Effect of Forest Fragmentation on Tick Infestations of Birds and Tick Infection Rates by *Rickettsia* in the Atlantic Forest of Brazil

Maria Ogrzewalska,[1,4] Alexandre Uezu,[2] Clinton N. Jenkins,[3] and Marcelo B. Labruna[1]

[1]*Faculty of Veterinary Medicine, University of São Paulo, São Paulo, SP, Brazil*
[2]*Instituto de Pesquisas Ecológicas (IPÊ), Nazaré, Paulista, SP, Brazil*
[3]*Department of Biology, North Carolina State University, Raleigh, NC*
[4]*Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia,
Universidade de São Paulo, Av. Prof. Dr. Orlando Marques de Paiva 87, São Paulo, SP 05508-270, Brazil*

Abstract: Habitat loss and modifications affect biodiversity, potentially contributing to outbreaks of infectious diseases. We evaluated if the patch sizeinfragmented areas of Atlantic Forest in southeastern Brazil influences the diversity of forest birds and consequently the prevalence of ticks on birds and the rickettsial infection of these ticks. During 2 years, we collected ticks from birds in 12 sites: four small forest patches (80–140 ha), four large ones (480–1,850 ha), and four forest control areas within the much larger Morro do Diabo State Park (~36,000 ha). A total of 1,725 birds were captured (81 species, 24 families), from which 223 birds were infested by 2,339 ticks of the genus *Amblyomma*, mostly by the species *A. nodosum*. Bird diversity and richness were higher in larger than smaller forest fragments. The prevalence of ticks on birds was inversely correlated with bird diversity and richness. Among 174 *A. nodosum* tested for rickettsial infection by polymerase chain reaction, 51 were found to be infected by *Rickettsia bellii* or *Rickettsia parkeri*. However, tick infection rates by *Rickettsia* spp. were not statistically different between forest patch sizes. The higher prevalence of ticks on birds in degraded patches might be caused by a dominance of a few generalist bird species in small patches, allowing an easier transmission of parasites among individuals. It could also be related to more favorable microclimatic conditions for the free-living stages of *A. nodosum* in smaller forest fragments.The higher burden of ticks on birds in smaller forest fragments is an important secondary effect of habitat fragmentation, possibly increasing the likelihood of Rickettsia contagion.

Keywords: ticks, birds, rickettsia, Atlantic Forest, fragmentation, biodiversity

INTRODUCTION

Habitat loss and fragmentation are primary causes of biodiversity loss world wide, increasing the risk of species extinction (Fahrig 2003; Fischer and Lindenmayer 2007). Besides this direct consequence, these processes may also trigger other outcomes, such as the emergence of infection diseases (Daszak et al. 2000; Dobson and Foufopoulus 2001; Daszak and Cunningham 2003; Sutherst 2004; Jones et al. 2008). This potentially disrupts an important ecosystem service, the maintenance of a low and balanced level

Correspondence to: Maria Ogrzewalska, e-mail: mogrzewalska@gmail.com

of parasites and pathogens in undisturbed ecosystems (Ostfeld and LoGiudice 2003; Keesing et al. 2010).

We attempted to verify the effect of forest fragmentation on bird diversity, the consequential effect of changes in the prevalence of ticks infesting wild birds, and the rickettsial infection in these ticks. Our hypotheses were that: (i) forest fragment size influences bird diversity and richness; (ii) the richness and the diversity of birds, and the abundance of common bird species, influence the prevalence of ticks on birds; (iii) the richness and diversity of birds influence the prevalence of *Rickettsia* infection rates of ticks.

Our study area was the Atlantic Forest landscape, specifically the Pontal do Paranapanema region in southeastern Brazil. The Atlantic Forest is the fourth most threatened biodiversity hotspot in the world (Mittermeier et al. 1999; Myers et al. 2000), with only 11–16% of the original biome remaining (Ribeiro et al. 2009). Not only is the loss of biodiversity critical in this biome, but so is the (re)emergence of diseases provoked by the intense alteration of this forest and the wide interface of people living around the forest.

A fragmented landscape differs from a continuous one by presenting: (i) a larger amount of edge, which increases interactions among adjacent ecosystems (both natural and anthropogenic) and causes biotic and abiotic changes in both directions across the boundary (Murcia 1995); (ii) a lower connectivity, resulting in higher isolation of species populations among fragments (Tischendorf and Fahrig 2000); and (iii) an increased local probability of species extinction (Ferraz et al. 2007). While the first characteristic is a primary factor in the dissemination of diseases among native and domestic animals, and to humans, the latter two are considered the main factors for biodiversity loss (Fahrig 2003; Uezu et al. 2005; Fischer and Lindenmayer 2007), particularly in a landscape with very low habitat cover (e.g., <30%). More specifically, in a poorly connected landscape, patch size is the primary explanatory variable of species richness, abundance, and composition (Andrén 1994; Pardini et al. 2010; Uezu and Metzger 2011).

Habitat loss and fragmentation might contribute to outbreaks of infectious diseases via both direct and indirect mechanisms. They can do so directly by creating favorable conditions for the proliferation and spread of disease vectors or of species hosts that are competent reservoirs for a pathogen. Examples include an increase of malaria vectors (*Anopheles* sp.) because of optimal microclimate conditions in deforested areas (Yasuoka and Levins 2007), and the

increase in songbird exposure to West Nile Virus by increasing levels of urbanization (Bradley et al. 2008).

Habitat loss and fragmentation can also contribute to disease outbreaks indirectly through the loss of biodiversity. It has been suggested that biodiversity plays an important role in decreasing the prevalence of infection disease among hosts due to the dilution effect (Ostfeld and Keesing 2000). In undisturbed ecosystems, biodiversity tends to be greater and more evenly distributed. If a community includes both competent and incompetent reservoir hosts, the potential hosts that are poor reservoirs will dilute the chances of the pathogen dissemination. In contrast, in a disturbed community there may be a bias in the species composition toward the dominance of a few generalist species. If these species are competent hosts, pathogens will spread more easily (Ostfeld and Keesing 2000).

The dilution effect was first proposed for Lyme Disease, also a tick-borne disease (Ostfeld and Keesing 2000). Since then, several studies using simulation models, and experimental and empirical data, have revealed details of this mechanism of disease regulation (Ostfeld and Keesing 2000; Ostfeld and LoGiudice 2003; Keesing et al. 2010). More recently, this effect was also confirmed to explain the prevalence of other diseases, such as the West Nile virus (Ezenwa et al. 2006; Swaddle and Calos 2008; Allan et al. 2009), hantavirus (Clay et al. 2009; Suzán et al. 2009), and schistosomiasis (Johnson et al. 2009).

While the last few decades have seen a substantial improvement in our knowledge about the role of biodiversity in disease prevalence, the majority of studies have been in temperate zones and there is a lack of research in tropical areas. This is critical, as it is the tropics where biodiversity concentrates and at the same time where habitat destruction has been most intense, leading to a high risk of emerging disease hotspots (Sutherst 2004; Jones et al. 2008).

Ticks (Acari: Ixodidae) transmit a huge variety of pathogens and are among the most important vectors of diseases affecting humans and livestock (Jongejan and Uilenberg 2004). Some ticks parasitize a wide variety of hosts, while others are extremely selective and feed solely on one host species (Oliver 1989). For many tick species, the adult stages are more selective for larger sized-mammals, while immature stages (larvae and nymphs) parasitize a wide range of small animals, such as reptiles, rodents, and birds (Hoogstraal 1961; Oliver 1989). Birds also play important roles as carriers of infected ectoparasites and can distribute them within and between continents (Hubalek 2004), which is one mechanism responsible for the wide geographic distributions

of certain tick-borne pathogens (Anderson et al. 1986; Elfving et al. 2010; Hildebrandt et al. 2010).

In South America, the most important tick-borne zoonotic disease is Rocky Mountain spotted fever (RMSF), which is caused by the bacterium *Rickettsia rickettsii* (Labruna 2009). Bacteria of the genus *Rickettsia* are obligate intracellular organisms that infect invertebrate hosts worldwide (Raoult and Roux 1997; Perlman et al. 2006). RMSF was first recognized in Brazil in 1929, in the state of São Paulo (Dias and Martins 1939). After a relatively quiet period during the 1960s and 1970s, the disease reemerged in the state and became a serious public health problem of much greater magnitude. During the past two decades, approximately 350 laboratory-confirmed cases (case-fatality rate ≈30%) have been reported in southeastern Brazil (Labruna 2009). The bacterium *R. rickettsii* is transmitted in Brazil by the ticks *Amblyomma cajennense* and *Amblyomma aureolatum,* especially in areas of contact between anthropogenic and natural ecosystems. However, other *Amblyomma* ticks seem to be involved in the transmission of other rickettsiae, with several other *Rickettsia* species with suspected or confirmed pathogenicity to humans being recently reported to infect ticks in South America (Labruna 2009; Spolidorio et al. 2010).

MATERIALS AND METHODS

Study Site

'Pontal do Paranapanema' is in the extreme western part of the Atlantic Forest region, one of the most threatened of the global biodiversity hotspots (Myers et al. 2000). It covers an area of ∼245,000 ha in the state of São Paulo (22°53′S, 52°09′W, Fig. 1). The process of deforestation in the region is relatively recent, beginning about 50 years ago, but only 17% of the original biome remains in a matrix composed mainly of pastures and sugar cane plantations (Leite 1998). The mean annual precipitation is 1,500 mm. The original forest type is considered to be seasonal semideciduous (Oliveira-Filho and Fontes 2000), being shaped by two extreme climatic seasons, a tropical season with intense rain during spring and summer, and a subtropical dry season with physiologic drought caused by low temperatures during autumn–winter (Veloso et al. 1991). An important element in the landscape is the Morro do Diabo State Park, which covers about 36,000 ha, and constitutes the largest Atlantic Forest remnant in the interior of the

state of São Paulo (Leite 1998). In the proximity of this park, there are many forest remnants that vary in size from two to 2,000 ha (Ditt 2002).

Landscape Parameters and Study Design

Previous studies in the region considered the whole community of birds from 28 areas (21 forest patches and seven forested control areas inside the Morro do Diabo State Park) and revealed that bird richness and diversity are particularly influenced by forest patch size (Uezu and Metzger 2011). For the present study, we selected 12 sample sites: four small forest patches (80–140 ha; S1, S2, S3, S4), four large forest patches (480–1,850 ha; L1, L2, L3, L4), and four control areas within the Morro do Diabo State Park (∼36,000 ha; C1, C2, C3, C4) (Fig. 1). All sample sites were forest with a similar vegetation structure and were a subset of the sample units previously studied (Uezu and Metzger 2011). All sites were within a similar matrix of pasture and sugar cane plantations except for the control sites, which were within the large forested area of Morro do Diabo State Park. Patch sizes were obtained from maps generated from satellite image classification (A. Uezu, unpublished data).

Capture Events

Forest birds were caught every season from December 2004 to December 2006 in each sampled forest area. In total, there were eight capture events per forest area at 3-month intervals. For each capture event, 15 mist nets (12 m long × 2 m wide, 36 mm mesh) were deployed in the forest during the day, starting between 6:30 and 7:30 am depending on the season, totaling 23,688 net-hours. To avoid potentially strong influences from the edge-effect, nets were deployed a minimum distance of 50 m inside from the forest edge. We identified captured birds to the species, banded, and examined them for the presence of ticks, which were all collected for later identification.

During each capture period, collection of free-living ticks was carried out in the same forest patch of bird captures using the standard method of dragging a piece of white flannel (100 cm × 50 cm) along 1,000 m of forest trails as previously described (Ogrzewalska et al. 2009a). For counting purposes, nymphs and adult ticks were counted individually, whereas larvae were counted as the number of clusters (i.e., each larval cluster collected from the environment was considered a single unit).
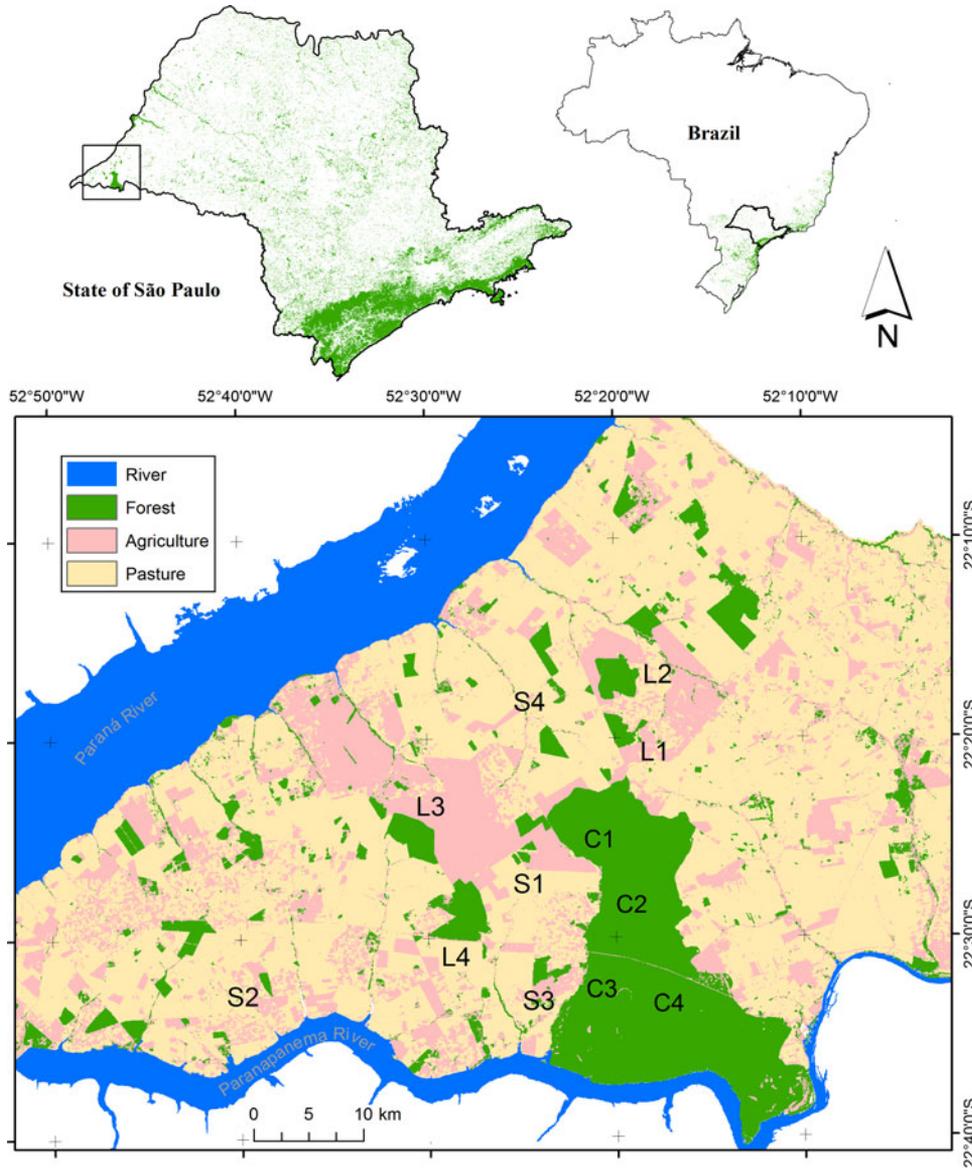
Figure 1. Study area of Pontal do Paranapanema Region, SP, Brazil. Small patches: S1, S2, S3, S4; large patches: L1, L2, L3, L4; Control areas inside the Morro do Diabo State Park: C1, C2, C3, C4.

Tick identification was performed as described in Ogrzewalska et al. (2009a), who reported raw data on the ticks collected in the present study. In addition, the rickettsial infection of these ticks was previously reported in Ogrzewalska et al.(2009b), who applied polymerase chain reaction (PCR) and DNA sequencing for detection and identification of rickettsiae infecting the ticks (detailed techniques were described by the authors). Herein, we analyzed both these tick and rickettsial data in relation to forest fragmentation.

Data Analyses

For each sample site, we calculated the following parameters: bird richness, Shannon diversity index, bird abundance (number of individuals per 100 net-hours), and the percentage of bird individuals infested with ticks (number of infested birds/number of examined birds × 100, for each bird species).

To test for the effect of habitat reduction on bird species, we used the Kruskal–Wallis test with the a posteriori Dunn's Test. For birds that occurred in more than seven sampled areas, if the abundance was higher in control and large fragments in relation to small fragments, we considered the species affected by fragmentation. If the abundance was constant between different size categories, we considered the species not affected. If the abundance was higher in small fragments, we considered the species to have benefited. For species that occurred in four to seven sampled areas, categorization was made based on presence/

absence. Species that occurred only in control and large fragments were considered affected. If species were present in all patch size classes, they were considered not affected. When species were present only in small areas, they were considered to have benefited. For species that occurred in less than four sampled areas, or if it was not possible to determine their response through the criteria mentioned above, the effect of fragmentation was determined based on data from previous studies in the same region (Uezu and Metzger 2011).

To compare the richness and diversity of birds and the prevalence of ticks on birds between the size categories, we again used the Kruskal–Wallis test with the a posteriori Dunn's test.

The influence of the richness and diversity of birds, and abundance of the most common birds infested by ticks (*Conopophaga lineata* and *Thamnophilus pelzelni*) on the prevalence of ticks in each studied area was verified through simple linear regressions.

To test for the influence of forest fragment size on the abundance of ticks in the environment, we used the same procedures described above for birds. However, for these regression analyses, values from control areas were averaged and represented by a single point as the control areas were located inside the same continuous forest. Tick infection rates by *Rickettsia* were compared between the forest fragments using a Chi-square test.

RESULTS

We captured 1,725 birds representing 80 species from 24 families within 10 orders, excluding recaptures from the same day. The most abundant species was *T. pelzeni* (317 individuals), followed by *C. lineata* (150 individuals) and *Arremon flavirostris* (147 individuals) (Table 1).

Bird species showed different responses to fragmentation, which we categorized into three classes: (1) sensitive species that were present only in the control areas and/or large fragments; (2) unaffected species whose abundance did not change between sampled areas; and (3) benefited species that increased their abundance in smaller fragments. Species such as *Corythopis delalandi*, *Malacoptila striata*, *Schiffornis virescens*, *Xenops rutilans*, *Nonnula rubecula*, *Hemitriccus orbitatus*, and *Chiroxiphia caudata* were present only in control areas and/or in large fragments and thus were considered sensitive to fragmentation. Similarly, species such as *Baryphthengus ruficapillus*, *Pipra fasciicauda*,

Table 1. The number of individuals caught of the 20 most abundant bird species caught by mist nets from December 2004 to December 2006

Species	Frequency
Thamnophilus pelzeni	317
Conopophaga lineata	150
Arremon flavirostris	147
Pipra fasciicauda	145
Platyrinchus mystaceus	94
Cnemotriccus bimaculatus	78
Baryphthengus ruficapillus	76
Basileuterus culicivorus	69
Sittasomus griseicapillus	66
Basileuterus flaveolus	64
Corythopis delalandi	64
Dysithamnus mentalis	43
Leptopogon amaurocephalus	27
Leptotila verreauxi	21
Pyriglena leucoptera	21
Malacoptila striata	19
Picumnus albosquamatus	19
Thalurania glaucopis	18
Thamnophilus caerulescens	18
Turdus amaurochalinus	18

and *Platyrinchus mystaceus* were significantly ($P < 0.05$) more abundant in control than in smaller fragments. Other species, such as *T. pelzelni, C. lineata, A. flavirostris, Basileuterus culicivorus, Basileuterus flaveolus, Sittasomus griseicapillus, Dysithamnus mentalis, Leptotila verreauxi,* and *Pyriglena leucoptera* maintained the same abundance between sampled areas, being not significantly affected by fragmentation ($P > 0.05$) (Fig. 2). Conversely, species such as *Cnemotriccus fuscatus, Hemitriccus margaritaceiventer, Formicivora rufa* and *Cyanocorax chrysops* benefited from a fragmented landscape, having significantly higher abundances in smaller patches ($P < 0.05$).

Bird diversity was influenced by fragment size, being lower in smaller fragments [$H_{(12.2)} = 6.27$; $P = 0.043$] (Fig. 3). Similarly, a tendency for lower richness in smaller fragments was also observed (Fig. 3), but the P value was slightly higher than assumed criteria [$H_{(12.2)} = 5.88$; $P = 0.053$].

A total of 223 (13%) birds were found infested by 1, 800 larvae and 539 nymphs of the genus *Amblyomma*. Identification of these ticks were as follows: *Amblyomma nodosum* (58 larvae, 394 nymphs), *Amblyomma longirostre*
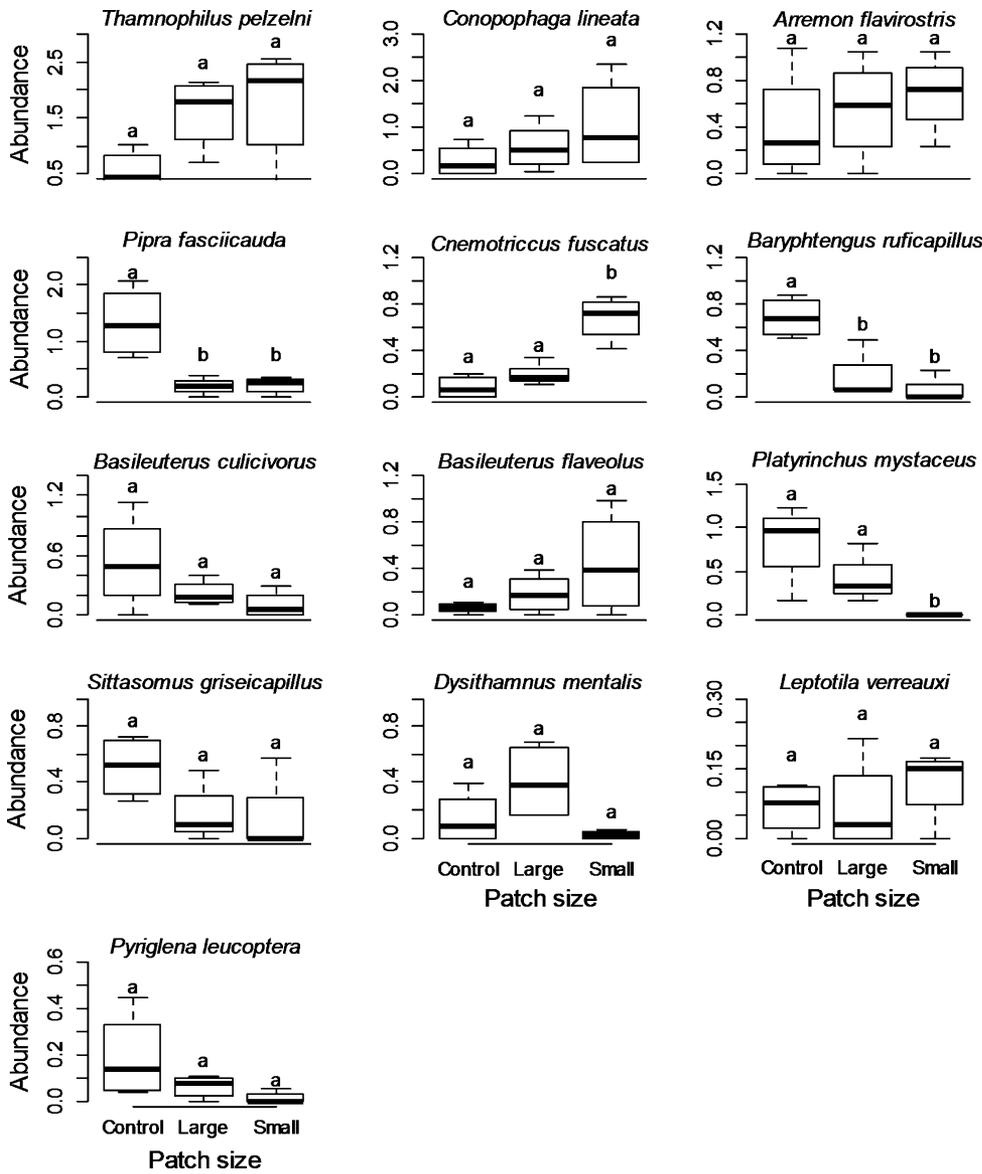
Figure 2. Variability by patch size in the abundance of the 13 most common birds caught by mist nets. The limits of the *boxes* indicate the first and third quartile and *lines* indicate the minimum and maximum abundance. Results with *different letters* indicate statistically significant differences (i.e., $P < 0.05$).
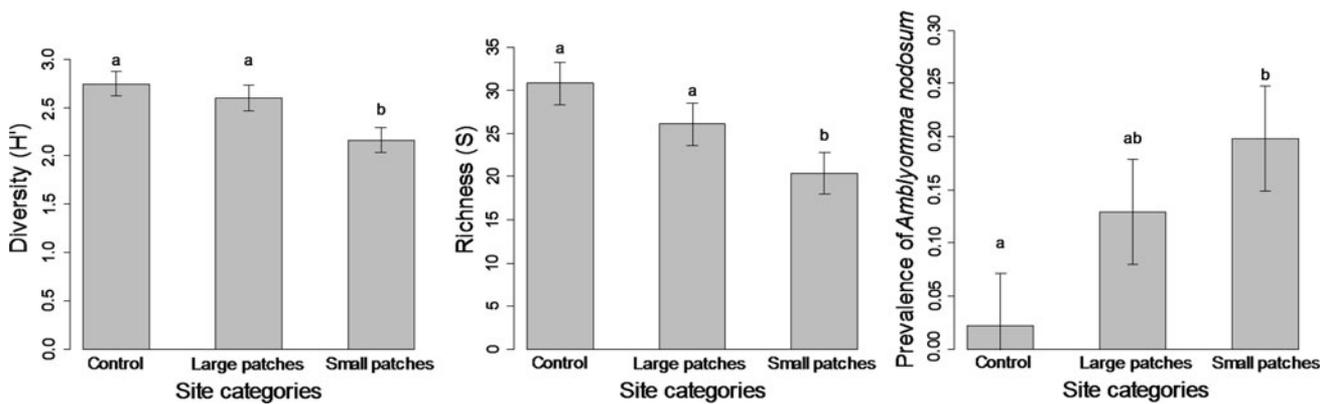

Figure 3. Variation in bird diversity, richness and in the prevalence of *Amblyomma nodosum* among sampled sites in Pontal do Paranapanema region. *Lines* indicate standard errors. Results with *different letters* indicate statistically significant differences (i.e., $P < 0.05$).

(4 larvae, 3 nymphs), *A. cajennense* (12 nymphs), *Amblyomma calcaratum* (2 nymphs), *Amblyomma coelebs* (11 larvae, 12 nymphs), *Amblyomma ovale* (5 larvae, 5 nymph), and *Amblyomma naponense* (1 nymph). A total of 1,722 larvae and 110 nymphs collected from birds could not be identified to species and were regarded as *Amblyomma* sp. Detailed data are presented elsewhere (Ogrzewalska et al. 2009a).

We found an inverse relationship between the prevalence of ticks on birds and the diversity and richness of birds ($R^2 = 0.51$; $P = 0.032$ and $R^2 = 0.44$; $P = 0.052$, respectively). When diversity and richness were higher, the prevalence of ticks was lower (Fig. 4).

We also found a positive relationship between the prevalence of ticks on birds and the abundance of *C. lineata* ($R^2 = 0.39$; $P = 0.03$; Fig. 4), although this pattern was not present for *T. pelzelni* ($R^2 = 0.01$; $P = 0.74$).

The prevalence of the most common tick to parasitize birds, *A. nodosum*, was influenced by the size of forest fragments, being higher in small fragments [$H_{(12.2)} = 6.64$; $P = 0.036$; Fig. 3]. We observed that *A. cajennense* and *A. longirostre* occurred in all three classes of patch size, while *A. coelebs*, *A. calcaratum,* and *A. naponense* occurred only in control and/or in large fragments, and *A. ovale* was more frequent in small fragments (Table 2). Since the occurrence of other tick species on birds was verified in only a few forest fragments, the effect of fragmentation on these ticks could not be tested statistically.

Among ticks collected from the environment *A. cajennense* was the most abundant, represented by 227 adults, 1,992 nymphs, and 31 larval clusters, representing 65.5% of all field-collected ticks. Other free-living species, *A. coelebs, A. naponense, A. brasiliense,* and *Haemaphysalis juxtakochi*, occurred only occasionally, representing 4.2% of all identified ticks. The remaining 30.3% of the free-living ticks were not identified to species and were regarded as *Amblyomma* sp. *A. cajennense* occurred in all sampled areas with the exception of one small fragment (P2). However, its abundance varied significantly, being higher in control and large fragments ($R^2 = 0.70$; $P < 0.01$; Fig. 4). *A. coelebs, A. brasilense,* and *A. naponense* occurred more frequently in control and large fragments, and sporadically in small fragments, while *H. juxtakochi* occurred in small numbers in fragments of different size categories (Table 2).

Among 174 nymphs of *A. nodosum* that were tested for rickettsial infection, 41 (23.6%) were found infected with *Rickettsia parkeri* strain NOD and 10 (5.7%) with *Rickettsia bellii*. Detailed data about molecular characterization and isolation of these bacteria from the *A. nodosum* ticks have
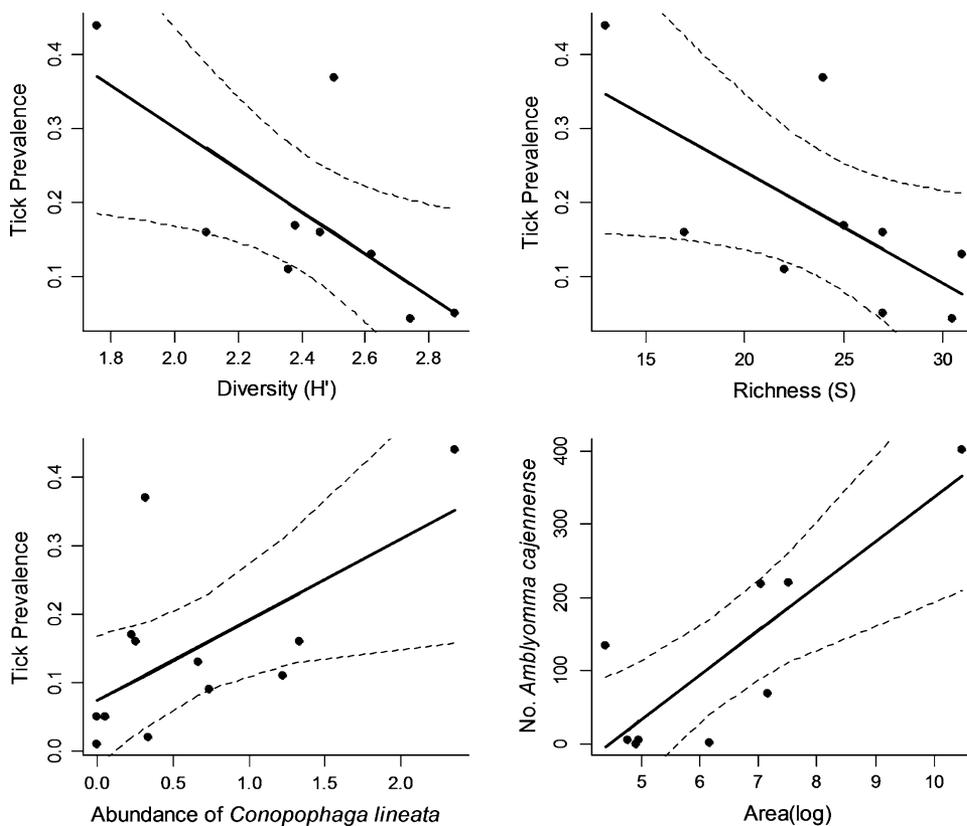

Figure 4. Variation in tick prevalence on birds according to bird species diversity and richness, abundance of *Conopophaga lineata* and abundance of *Amblyomma cajennense* among sampled sites in Pontal do Paranapanema region. *Dashed lines* show 95% confidence intervals.

Table 2. Number of ticks collected on birds or from the environment in three different patch size areas (Control, Large, and Small) in the Pontal do Paranapanema region, Brazil

Ticks species	Areas					
	Control		Large		Small	
	Bird	Environment	Bird	Environment	Bird	Environment
A. nodosum	25N	–	91N	–	278N, 58L	–
A. coelebs	2N, 11L	5A, 28N, 4C	10N	2A, 14N, 2C	–	1A, 5N
A. longirostre	1L	–	3L	–	3N	–
A. cajennense	8N	131A, 1443N, 24C	2N	78A, 426N, 6C	2N	18A, 123N, 1C
A. ovale	–	–	2N	–	3N, 5L	–
A. naponense	–	1A, 25N, 2C	1 N	4A, 7N	–	2A, 2N
A. calcaratum	2N	–	–	–	–	–
A. brasilense	–	4A, 13N, 4C	–	2A, 7N	–	3A, 2N
Amblyomma spp	9 N	490N, 26C	89N, 1661L	299N, 5C	12 N, 61L	221N, 1C
H. juxtakochi	–	2N	–	1 N	–	1 N
Total	46N, 12L	141A, 2001N, 60C	195N, 1664L	86A, 754N, 13C	298N, 124L	24A, 354N, 2C

A adult ticks, *N* nymphs, *L* larvae, *C* larval clusters, – no tick.

Table 3. Number of *Amblyomma nodosum* nymphs infected by *Rickettsia parkeri* or *Rickettsia bellii* according to the fragment size where the ticks were collected on birds in the Pontal do Paranapanema region, Brazil

Areas	No. of infected nymphs/No. tested (prevalence)	
	Rickettsia parkeri	*Rickettsia bellii*
Control	2/6 (33.3)	0/6 (–)
Large	6/38 (15.8)	3/38 (7.9)
Small	33/130 (25.4)	7/130 (5.4)
Total	41/174 (23.6)	10/174 (5.7)

been reported elsewhere (Ogrzewalska et al. 2009b). *R. parkeri* was found in 33.3, 15.8, and 25.8% of the nymphs from control, large, and small fragments, respectively. These infection rates were statistically similar (χ^2 = 1.83; DF = 2; P = 0.400). *R. bellii* was found in 7.9 and 5.5% of the nymphs from the large and small fragments, respectively (χ^2 = 0.33; DF = 1; P = 0.565). It was not found in the control area (Table 3).

DISCUSSION

During the last 50 years, the study region of Pontal do Paranapanema has suffered dramatic deforestation. The landscape is now composed predominantly of pastures and sugar cane plantations, with less than 20% of the original forest cover left (Leite 1998). Such changes cause drastic habitat fragmentation, leading to isolation and a decrease of animal populations (Fahrig 2003). In other regions, such ecological modifications are known to have altered the relationships between vertebrate hosts and their parasites, affecting their behaviors, movements, densities, and consequently influencing the transmission of vector-borne pathogens (Ostfeld and Keesing 2000).

In the present study, we verified that the diversity and richness of wild bird species were lower in smaller forest fragments, a finding that is in concordance with a previous study conducted in the same region (Uezu and Metzger 2011) and with other studies of avifauna (e.g., Willis 1979; Ribon et al. 2003; Uezu et al. 2005). Some more vulnerable bird species survive only in larger forest remnants, with the small and isolated fragments having become too small to sustain local populations and the birds being unable to cross the non-habitat portion of the landscape to maintain dispersal with other populations, leading to local extinction (Fahrig 2003).

Ticks are also affected by forest fragmentation. Considering the most abundant tick species found on birds (*A. nodosum*) or in the environment (*A. cajennense*), our results showed that the abundance of *A. nodosum* on birds was significantly higher in the fragmented landscape, while the abundance of *A. cajennense* on the vegetation was

significantly lower. Previous studies with other ticks species in other biomes showed that the abundance of the main mammalian hosts has a direct effect on tick abundance. In other words, the higher the host abundance, the higher the chances that ticks will find the hosts to complete their life cycle, increasing the tick population (Randolph 2004; Deblinger et al. 1993; Ostfeld and Keesing 2000; Allan et al. 2003; Rizzoli et al. 2009).

Amblyomma nodosum is a poorly studied Neotropical tick, for which the adult stage feeds chiefly on the anteaters *Tamandua tetradactyla* and *Myrmecophaga tridactyla*, with understory Passeriformes birds serving as hosts for the immature stages (Labruna et al. 2007; Ogrzewalska et al. 2009a). In the present study, the birds *T. pelzelni* and *C. lineata* were the most frequent hosts of immature *A. nodosum* in all fragments sampled (small, large, and control areas). Although the overall abundance of these two bird species were not significantly different between control, large, and small areas (Fig. 2), a total of 147 *T. pelzelni* and 84 *C. lineata* were captured in small fragments during the study, in contrast to only 43 *T. pelzelni* and 19 *C. lineata* captured in the control areas. Thus, it is possible that higher host availability in the small fragments could have accounted for the higher abundance and prevalence of *A. nodosum* infestations on birds in these small forest areas. The prevalence of ticks on birds is also significantly correlated with the abundance of *C. lineata*, supporting this conjecture.

The two main hosts of adult *A. nodosum* ticks, *M. tridactyla* and *T. tetradactyla*, were known to occur in our study region (Lima 2009). In this case, different abundance of these mammals could also have affected our results. It is possible that abundance of anteaters was higher in small fragments, due to higher food availability (ants and termites) or because of the lack of predators like ocelots and jaguars (Redford and Eisenberg 1989), which are the first to disappear in fragmented landscapes (Noss et al. 2005). However, even though *M. tridactyla* and *T. tetradactyla* are found in all Brazilian biomes, where they are able to use many types of vegetation from tropical rainforest to savannahs, we are aware of no studies concerning their abundance. Therefore, it is not possible to confirm if their populations were influenced by habitat fragmentation or if *A. nodosum* was affected by anteater abundance.

Another factor that could have affected the distribution of ticks is the microclimate of the fragments. The ticks found in the present study take only one blood meal per life stage, as larva, as nymph, and as adult. Each feeding stage

takes no more than a few days on the host, when the ticks drop off to develop to the next stage, which might take weeks to months between successive stages. Thus, despite being parasites, these ticks spend most of their life cycle in the environment, where they also take time to search for their hosts and are strongly influenced by environmental conditions (Oliver 1989; Randolph 2004). Successful development of free-living stages is affected primarily by temperature and humidity, which have been shown to affect tick abundance (Randolph 2004). Although there are no data on optimal microclimatic requirements for *A. nodosum*, it is suspected that this species, which commonly parasitizes anteaters in the Cerrado biome (a Brazilian savannah less humid than rainforest), finds more optimal conditions in smaller and degraded forest patches. Such smaller fragments are strongly influenced by their surroundings and, because of a less dense canopy, the temperature should be higher and the humidity lower when compared with the conditions of the original Atlantic Forest.

The most common tick found in the environment, *A. cajennense*, feeds on a wide range of animals, although larger mammals such as tapirs (*Tapirus terrestris*) and peccaries (*Tayassu* spp.) are preferable for adult ticks (Aragão 1936; Estrada-Pena et al. 2004). The highest abundance of this tick species was observed in the control areas, while the species seems to vanish from small and isolated fragments, suggesting that its principal wild hosts (tapirs and peccaries) have already disappeared from these small fragments. This disappearance was recently confirmed in a field study on peccaries in the same forest fragments of the present study (Nava 2009). A similar tendency was observed for other tick species found in the environment, such as *A. coelebs* that parasitizes mainly tapirs, and *A. naponense* and *A. brasiliense* that parasitize chiefly peccaries (Labruna et al. 2005; Szabo et al. 2009). These results confirm that these medium to large-sized mammals are also disappearing from small forest fragments, perhaps leading to the local co-extinction of their ticks. In this case, our results confirm that questing ticks can be used as bio-indicators of wild fauna (Labruna and Guglielmone 2009).

In the studied region, 5.7 and 23.6% of *A. nodosum* nymphs collected on birds were infected with *R. parkeri* and *R. bellii*, respectively (Ogrzewalska et al. 2009b). *R. bellii* is the most commonly found rickettsia in Neotropical ticks, and there is no evidence that it is pathogenic for humans or animals (Labruna 2009). On the other hand, *R. parkeri* is

responsible for spotted fever cases in humans in the United States (Paddock et al. 2004), Uruguay (Conti-Díaz et al. 2009), and Brazil (Spolidorio et al. 2010). In Brazil, different strains of *R. parkeri* have been reported in ticks, including the strain NOD, also detected in *A. nodosum* ticks from our study region (Ogrzewalska et al. 2009b).

CONCLUSIONS

The proportion of *Rickettsia*-infected ticks found in our study was not exceptionally high. However, forest fragmentation showed a significant effect on tick abundance and on bird richness and abundance. While there did not appear to be a significant change in the actual rates of Rickettsia infection in *A. nodosum* ticks, the greater absolute abundance of ticks likely increases the risk of Rickettsia contagion. These results probably occurred because regardless of the fragment size, the *A. nodosum* population tended to feed mostly on the same host species. Additionally, it is possible that the main perpetuation route of *R. parkeri* strain NOD among *A. nodosum* ticks is through vertical transmission, as reported for other rickettsial strains in other tick species (Parola et al. 2005). Thus, horizontal transmission via vertebrate hosts could be of little importance for this rickettsia. We conclude that the higher burdens of immature *A. nodosum* ticks on wild birds in small forest remnants, and the tendency of other ticks typical for ungulates (especially *A. cajennense*) to disappear from these small fragments, are important effects of habitat fragmentation, showing the imbalance between the environment–host–parasite relationship.

ACKNOWLEDGMENTS

REFERENCES

Allan BF, Keesing F, Ostfeld RS (2003) Effect of forest fragmentation on Lyme disease risk. *Conservation Biology* 17:267–272

Allan BF, Langerhans RB, Ryberg WA, Landesman WJ, Griffin NW, Katz RS, Oberle BJ, Schutzenhofer MR, Smyth KN, de St Maurice A, Clark L, Crooks KR, Hernandez DE, McLean RG, Ostfeld RS, Chase JM (2009) Ecological correlates of risk and incidence of West Nile virus in the United States. *Oecologia* 158:699–708

Anderson JF, Johnson RC, Magnarelli LA, Hyde FW (1986) Involvement of birds in the epidemiology of the Lyme disease agent *Borreliaburgdofreri*. *Infection and Immunity* 2:394–396

Andrén H (1994) Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. *Oikos* 71:355–366

Aragão HB (1936) Ixodidas brasileiros e de alguns paizes limitrophes. *Memorias do Instituto Oswaldo Cruz* 31:759–843

Bradley CA, Gibbs SE, Altizer S (2008) Urban land use predicts West Nile virus exposure in songbirds. *Ecological Applications* 18:1083–1092

Clay CA, Lehmer EM, Jeor SS, Dearing MD (2009) Testing mechanisms of the dilution effect: deer mice encounter rates, Sin Nombre virus prevalence and species diversity. *EcoHealth* 6:250–259

Conti-Díaz IA, Moraes-Filho J, Richard C, Pacheco MB, Labruna RC (2009) Serological evidence of *Rickettsia parkeri* as the etiological agent of rickettsiosis in Uruguay. *Revistado Instituto de MedicinaTropical de São Paulo* 51:337–339

Daszak P, Cunningham AA (2003) Anthropogenic change, biodiversity loss, and a new agenda for emerging diseases. *Journal of Parasitology* 37–41:2003

Daszak P, Cunningham AA, Hayatt AD (2000) Emerging infectious diseases of wildlife. Threats to biodiversity and human health. *Science* 287:443–449

Deblinger RD, Wilson ML, Rimmer DW, Spielman A (1993) Reduced abundance of immature *Ixodes dammini* (Acari: Ixodidae) following incremental removal of deer. *Journal of Medical Entomology* 30:144–150

Dias E, Martins AV (1939) Spotted fever in Brazil. A summary. *Journal of Tropical Medicine and Hygiene* 19:103–108

Ditt EH (2002) *Fragmentos florestais no Pontal do Paranapanema*, *1st ed.*, São Paulo: Annablume

Dobson A, Foufopoulus J (2001) Emerging infectious pathogens of wildlife. *Philosophical Transaction of The Royal Society of London* 356:1001–1012

Elfving K, Olsen B, Bergstrom S, Waldenstrom J, Lundkvist A, Sjostedt A, Mejlon H, Nilsson K (2010) Dissemination of spotted fever Rickettsia agents in Europe by migrating birds. *PLoS One* 5:e8572

Estrada-Pena A, Guglielmone AA, Mangold AJ (2004) The distribution and ecological 'preferences' of tick *Amblyomma cajennense* (Acari: Ixodidae), an ectoparasite of humans and other mammals in the Americas. *Annals of Tropical Medicine and Parasitology* 98:283–292

Ezenwa VO, Godsey MS, King RJ, Gupthill SC (2006) Avian diversity and West Nile virus: testing associations between biodiversity and infectious disease risk. *Proceedings of the Royal Society B: Biological Sciences* 273:109–117

Fahrig L (2003) Effects of habitat fragmentation on biodiversity. *Annual Review of Ecology and Systematics* 34:487–515

Ferraz G, Nichols JD, Hines JE, Stouffer PC, BierregaardJr RO, Lovejoy TE (2007) A large-scale deforestation experiment: effects of patch area and isolation on Amazon birds. *Science* 315:238–241

Fischer J, Lindenmayer DB (2007) Landscape modification and habitat fragmentation: a synthesis. *Global Ecology and Biogeography* 16:265–280

Hildebrandt A, Franke J, Meier F, Sachse S, Dorn W, Straube E (2010) The potential role of migratory birds in transmission cycles of *Babesia* spp., *Anaplasma phagocytophilum*, and *Rickettsia* spp. *Ticks and Tick-borne Diseases* 1:105–107

Hoogstraal H (1961) Migrating birds and their ectoparasites in relation to disease. *The East African Journal* 38:221–226

Hubalek Z (2004) An annotated checklist of pathogenic microorganisms associated with migratory birds. *Journal of Wildlife Diseases* 40:639–659

Johnson P, Lund TJP, Hartson RB, Yoshino T (2009) Community diversity reduces *Schistosoma mansoni* transmission and human infection risk. *Proceedings of the Royal Society B: Biological Sciences* 276:1657–1663

Jones KE, Nikkita G, Patel NG, Marc A, Levy MA, Adam Storeygard A, Deborah Balk D, Gittleman JL, Peter Daszak P (2008) Global trends in emerging infectious diseases. *Nature* 451:990–993

Jongejan G, Uilenberg G (2004) The global importance of ticks. *Parasitology* 129:3–14

Keesing F, Belden LK, Daszak P, Dobson A, Harvell CD, Holt RD, Hudson P, Jolles A, Jones KE, Mitchell CE, Myers SS, Bogich T, Ostfeld RS (2010) Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature* 468:647–652

Labruna MB (2009) Ecology of *Rickettsia* in South America. *Annals of New York Academy of Science* 1166:156–166

Labruna MB, Guglielmone AA (2009) Ticks of New World tapirs. *Tapir Conservation* 18:21–28

Labruna MB, Camargo LMA, Terrassini FA, Ferreira F, Schumaker TTS, Camargo EP (2005) Ticks (Acari: Ixodidae) from the state of Rondônia, western Amazon, Brazil. *Systematic & Applied Acarology* 10:17–32

Labruna MB, Sanfilippo LF, Demetrio C, Menezes AC, Pinter A, Guglielmone AA, Silveira LF (2007) Ticks collected on birds in the state of São Paulo. *Experimental and Applied Acarology* 43:147–160

Leite FJ (1998) *A ocupação do Pontal do Paranapanema*, Sao Paulo: Hucitec, Fundação UNESP

Lima F (2009) Estimativas de abundância e densidade populacional da jaguatirica através de modelos de marcação-recaptura: Estudo de caso nos remanescentes florestais do Pontal do Paranapanema. Dissertação (Mestrado em Zoologia dos Vertebrados), Pontifícia Universidade Católica de Minas Gerais: Belo Horizonte

Mittermeier RA, Myers N, Gil PR, Mittermeier CG (1999) *Hotspots: earth's biologically richest and most endangered terrestrial ecoregions*, Mexico City: CEMEX

Murcia C (1995) Edge effects in fragmented forests: implications for conservation. *Trends in Ecology and Evolution* 2:58–62

Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature* 403:853–858

Nava AFD (2009) Espécies sentinelas para a Mata Atlântica: as conseqüências epidemiológicas da fragmentação florestal no Pontal do Paranapanema. PhD Thesis, Universidade de São Paulo: São Paulo

Noss RF, Csuti B, Groom MJ (2005) Habitat fragmentation. In: *Principles of Conservation Biology, 3rd ed*, Groom MJ, Meffe GK, Carroll CR (editors), Massachusetts: Sinauer

Ogrzewalska M, Pacheco RC, Uezu A, Richtzenhain LJ, Ferreira F, Labruna MB (2009) Ticks (Acari: Ixodidae) infesting birds in an Atlantic Rain Forest Region of Brazil. *Journal of Medical Entomology* 46:1225–1229

Ogrzewalska M, Pacheco RC, Uezu A, Richtzenhain LJ, Ferreira F, Labruna MB (2009) Rickettsial infection in *Amblyomma nodosum* ticks (Acari: Ixodidae) from Brazil. *Annals of Tropical Medicine and Parasitology* 103:413–425

Oliveira-Filho AT, Fontes MAL (2000) Patterns of floristic differentiation among Atlantic Forests in southeastern Brazil and influence of climate. *Biotropica* 32:793–810

Oliver H Jr (1989) Biology and systematics of ticks (Acari: Ixodida). *Annual Review of Ecology and Systematics* 20:397–430

Ostfeld RS, Keesing F (2000) Biodiversity and disease risk: the case of Lyme disease. *Conservation Biology* 14:722–728

Ostfeld RS, Logiudice K (2003) Community disassembly, biodiversity loss, and the erosion of an ecosystem service. *Ecology* 84:1421–1427

Paddock CD, Summer JW, Comer JA, Zaki SR, Goldsmith CS, Goddard J, McLellan SLF, Tamminga CL, Ohl CA (2004) *Rickettsia parkeri*: a newly recognized cause of spotted fever rickettsiosis in the United States. *Clinical Infectious Diseases* 38:805–811

Pardini R, Bueno AA, Gardner TA, Prado PI, Metzger JP (2010) Beyond the fragmentation threshold hypothesis: regime shifts in biodiversity across fragmented landscapes. *PLoS One* 5:e13666

Parola P, Paddock CD, Raoult D (2005) Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. *Clinical Microbiology Reviews* 18:719–756

Perlman SJ, Hunter MS, Zchori-Fein E (2006) The emerging diversity of *Rickettsia*. *Proceedings of the Royal Society B: Biological Sciences* 273:2097–2106

Randolph SE (2004) Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. *Parasitology* 129:37–65

Raoult D, Roux V (1997) Rickettsioses as paradigms of new or emerging infectious diseases. *Clinical Microbiology Reviews* 10:694–719

Redford KH, Eisenberg JF (1989) *Mammals of the Neotropics. The Central Neotropics. Ecuador, Peru, Bolivia, Brazil*, Chicago, London: The University of Chicago Press

Ribeiro MC, Metzger JP, Martensen AC, Ponzoni FJ, Hirota MM (2009) The Brazilian Atlantic Forest: how much is left, and how is the remaining forest distributed? Implications for conservation *Biological Conservation* 142:1141–1153

Ribon R, Simon JE, Mattos GT (2003) Bird extinctions in Atlantic Forest fragments of Viçosa Region, Southeastern Brazil. *Conservation Biology* 17:1827–1839

Rizzoli A, Hauffe HC, Tagliapietra V, Neteler M, Rosa R (2009) Forest structure and Roe deer abundance predict tick-borne encephalitis risk in Italy. *PLoS One* 4:e4336

Spolidorio MG, Labruna M, Mantovani E, Brandao P, Richtzenhain L, Yoshinari N (2010) Novel spotted fever group rickettsiosis, Brazil. *Emerging Infectious Diseases* 16:521–523

Sutherst RW (2004) Global change and human vulnerability to vector-borne diseases. *Clinical Microbiology Review* 17:136–173

Suzán G, Marcé E, Giermakowski JT, Mills JN, Ceballos G, Ostfeld RS, Armién B, Juan M, Pascale JM, Yates TL (2009) Experimental evidence for reduced rodent diversity causing increased hantavirus prevalence. *PLoS ONE* 4(5):e5461. doi:10.1371/journal.pone.0005461

Swaddle J, Calos P (2008) Increased avian diversity is associated with lower incidence of human West Nile infection: observation of the dilution effect. *PLoS One* 3:e2488

Szabo MPJ, Labruna MB, Garcia MV, Pinter A, Castagnolli KC, Pacheco RC, Castro MB, Veronez VA, Magalha GM, Vogliotti ESA, Duarte JMB (2009) Ecological aspects of the free-living ticks (Acari: Ixodidae) on animal trails within Atlantic rainforest in south-eastern Brazil. *Annals of Tropical Medicine and Parasitology* 103:57–72

Tischendorf L, Fahrig L (2000) On the usage and measurement of landscape connectivity. *Oikos* 90:7–19

Uezu A, Metzger JPW (2011) Vanishing bird species in the Atlantic Forest: relative importance of landscape configuration, forest structure and species characteristics. Biodiversity and Conservation. doi:10.1007/s10531-011-0154-5

Uezu A, Metzger JPW, Vielliard JM (2005) The effect of structural and functional connectivity and patch size on the abundance of seven Atlantic Forest bird species. *Biological* 123:507–519

Veloso HP, Rangel-Filho AL, Lima LCA (1991) *Classificação da vegetação brasileira, adaptada a um sistema universal*, Rio de Janeiro: Fundação Instituto Brasileiro de Geografia e Estatística

Willis EO (1979) The composition of avian communities in the remanescent woodlots in southern Brazil. *PapéisAvulsos de Zoologia* 33:1–25

Yasuoka J, Levins R (2007) Impact of deforestation and agricultural development on anopheline ecology and malaria epidemiology. *American Journal of Tropical Medicine and Hygiene* 76:450–460