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Effect of deforestation on prevalence of avian haemosporidian parasites and mosquito abundance in a tropical rainforest of Cameroon

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ABSTRACT

Habitat change caused by deforestation can modify the interactions of many biotic and abiotic factors, and in turn influence patterns of diseases in wild birds. Whether deforestation directly or indirectly affects the prevalence of avian haemosporidian parasites through their hosts and/or vectors is still not well understood. We sampled understory bird communities (insectivorous, frugivorous, granivorous and nectarivorous birds) and mosquitoes in three habitats showing a gradient of deforestation (pristine forest, fragmented forest, and young palm oil plantation), to assess the effects of habitat changes on avian haemosporidian (*Plasmodium* and *Haemoproteus*) prevalence and its relationship to bird feeding guilds and mosquito abundance. Blood samples of 845 individual birds belonging to 85 species and 27 families were collected in the three habitat types and screened using microscopy and PCR. *Plasmodium* infections were detected in 136 individuals (16.09%) and varied significantly among habitat types while *Haemoproteus* infections were detected in 98 individuals (11.60%) and did not vary significantly among habitat types. However, the prevalence of *Plasmodium* and *Haemoproteus* in bird feeding groups varied significantly among habitats. Nectarivorous and granivorous birds had the highest *Plasmodium* and *Haemoproteus* prevalence, respectively. The abundance of mosquitoes varied significantly among habitat types and the prevalence of *Plasmodium* significantly and positively correlated with mosquito abundance in fragmented forest. This study highlights the importance of host and mosquito determinants in the transmission dynamics of avian *Plasmodium* and *Haemoproteus* infections following habitat changes. Selective logging favored an increase in the prevalence of *Plasmodium* in insectivores, the prevalence of *Haemoproteus* in nectarivores and the abundance of female mosquitoes while, the establishment of the palm oil plantation favored an increase in the prevalence of *Plasmodium* in granivores and *Haemoproteus* in nectarivores. Species feeding behavior is also an important determinant to consider for a better understanding of patterns of parasite infections in a changing environment.

1. Introduction

Tropical rainforests are the most diverse of habitats in their vegetation structure and composition. They support a diversity of faunal groups such as birds, reptiles, mammals, amphibians, and invertebrates, which depend on forest resources for their existence and survival (Zakaria et al., 2016). Unfortunately, these tropical forests are experiencing escalating human influence, altering their health and functions. Currently, the most prominent human-induced factors involved in land use alteration/degradation and loss include deforestation for agricultural expansion, industrial logging, gold mining and urbanization (Food and Agriculture Organization FAO, 2009; Lewis et al., 2015). The global rate of tropical deforestation is increasing rapidly, 500,000 square km of African land is estimated to be degraded, as the continent's population is expected to double to 2.5 billion by 2050 (Blaser et al., 2011). Cameroon accounts for the second highest rate of deforestation

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among the Congo Basin countries, with an equivalent of approximately 1% annual deforestation rate (FAO, 2015). Approximately 20% of tropical forests were affected by selective logging between 2000 and 2005, and more than 400 million hectares of tropical forest remain in permanent timber concessions (Blaser et al., 2011). Logging and agricultural expansion (mainly palm oil plantations) are a frequent gateway to habitat degradation and habitat conversion/loss in Cameroon. The consequences of such activities are diverse and consistently negative for ecosystem health. Deforestation directly alters many fundamental aspects of the ecosystem including landscape structure, the water cycle, local microclimates, as well as the physical and chemical properties of the soil (Sukhbaatar et al., 2019). It can also indirectly modify host-pathogen interactions, introducing novel pathogens into naïve populations, with an eventual increase in disease outbreaks in many vertebrates including humans (Sehgal, 2010; Gottwalt, 2013; Dutta and Dutta, 2015; Morris et al., 2016; Brock et al., 2019).

Avian blood pathogens, particularly the closely related genera *Plasmodium* and *Haemoproteus*, represent an excellent model in investigating the ecological determinants of vector-borne disease transmission dynamics under land use change (Knowles et al., 2009; Clark et al., 2014). *Plasmodium* sp., the causative agent of avian malaria, has been described as a potential cause of extinction and population decline in many bird species; they are known to reduce the fitness of the hosts and in some cases lead to death (Cannell et al., 2013; Paxton et al., 2016). Several studies have been carried out on the effects of human induced habitat changes on the prevalence and diversity of avian *Plasmodium* and *Haemoproteus* parasites. For example, Loiseau et al. (2010) reported a decrease in prevalence of parasites of malaria in the Olive sunbird, associated with increased forest disturbance in Ghana. When comparing pristine forests with deforested areas in Cameroon, Bonneaud et al. (2009) found that the prevalence of *Plasmodium* in birds was higher in the intact forested areas. Similar results were found in another study in the same region, where undisturbed habitats positively correlated with higher prevalence of *Haemoproteus* and *Leucocytozoon* parasites (Chasar et al., 2009). At the same time, other recent studies revealed that land use change has no effect on avian haemosporidian infections. For example, in a Brazilian Atlantic forest, although forest fragmentation was related to changes in host populations and vectors, no effect was found on avian blood parasites (Sebaio et al., 2012). Similarly, Rivero de Aguilar et al. (2018) reported no relationship between habitat fragmentation and the infection status of avian *Plasmodium* and *Haemoproteus* parasites. The effect of anthropogenic habitat changes on avian haemosporidian infections is still controversial. Therefore, more research is needed to better understand the effects of habitat modifications on avian haemosporidian parasites.

The prevalence of avian blood parasites has also been associated with a number of host biological and ecological characteristics. Studies on the effects of avian haemosporidians on bird biological and ecological traits focus more on traits such as sex, age, body mass, geographic range, foraging strata and nest types (Svensson-Coelho et al., 2013; Gonzalez et al., 2014; Lutz et al., 2015; Matthews et al., 2016; Gutiérrez-López et al., 2019). Less is known regarding the relationship between bird feeding habits and their susceptibility to haemosporidian parasites. Bird feeding guilds are important ecological determinants as they play functional roles through their trophic interactions with plants and insects. For example, frugivorous birds act on plant dispersal and recruitment, and this interaction is critical for the long-term resilience of forests undergoing anthropogenic change (Bregman et al., 2016). Insectivorous forest birds regulate the top-down control of herbivory by phytophagous insects. Previous studies of bird blood-borne parasites revealed that bird species belonging to insectivorous groups are more susceptible to *Haemoproteus* and *Plasmodium* infections (Laurence et al., 2013). These infections may cause the loss of some insectivorous birds and consequently affect their role in the ecosystem. In fact, loss of insectivorous species can lead to increased leaf damage, and hence both increased seedling mortality and reduced plant growth in degraded forests (Dunham, 2008). Research on this theme is still developing, with very few studies published, especially in a changing environment.

The transmission cycle and pathogenicity of *Plasmodium* and *Haemoproteus* parasites in birds are highly variable and depend on many factors. In fact, the parasite must be in the correct life stage, the bird must be available and susceptible to the parasite, the vector must be present and competent, and the environment must be permissive (Sehgal, 2015). The primary vectors of avian *Plasmodium* sp. are female mosquitoes of the genera *Culex*, *Aedes*, *Culiseta*, *Anopheles*, *Mansonia*, *Aedeomyia*, *Uranotaenia* and *Coquillettidia* (Valkiūnas, 2005; Ejiri et al., 2009; Njabo et al., 2009; Santiago-Alarcon et al., 2012; Okanga et al., 2013; Schmid et al., 2017; Abella-Medrano et al., 2018). *Haemoproteus* spp. are transmitted by hippoboscid flies in the case of the subgenus *Haemoproteus,* and ceratopogonid midges for the subgenus *Parahaemoproteus* (Valkiūnas, 2005). In sub-Saharan Africa, very few studies have explored the role of culicine mosquitoes in the transmission of avian *Plasmodium* (Njabo et al., 2009; Kimura et al., 2010; Okanga et al., 2013), and the possibility remains that new mosquito genera and species may still be identified as major and minor avian vectors. The few studies that integrated hosts-parasites and vectors in the same model revealed that avian *Plasmodium* prevalence was explained by vector-related variables, while parasite richness was mostly explained by vertebrate community-related variables (Ferraguti et al., 2018). To the contrary, Fecchio et al. (2017) observed that the variation in *Plasmodium* prevalence among bird species was not explained by avian ecological traits or mosquito abundance. Researchers are still far from understanding how the complex interactions among birds, avian blood parasites and vectors will be affected by anthropogenic environmental changes. Integrating host determinants and vectors in natural populations in different landscapes is timely and will bring additional information to clarify the effects of land use change on pathogenic infections.

To attempt to address these gaps, we sampled understory bird communities (insectivorous, frugivorous, granivorous and nectarivorous birds) and mosquitoes in three land use categories (pristine forest, fragmented forest, and young palm oil plantation) in the Talangaye rainforest, South West Region of Cameroon. We assessed the concomitant effects of habitat changes on mosquito abundance, avian haemosporidian infections, as well as the variation in these infections among bird feeding groups. We focus here on three questions: (i) Does the prevalence of *Plasmodium* and *Haemoproteus* vary among habitat types? (ii) Does habitat change affect the prevalence of *Plasmodium* and *Haemoproteus* among bird groups? (iii) Is *Plasmodium* prevalence related to mosquito abundance among habitat types? We hypothesized that: (i) the prevalence of *Plasmodium* and *Haemoproteus* will be highest in pristine forest (Chasar et al., 2009; Loiseau et al., 2010); (ii) insectivores will be more susceptible to *Plasmodium* and *Haemoproteus,* and infections in bird feeding groups will vary among habitat types (Laurence et al., 2013); (iii) mosquito abundance will positively correlate with *Plasmodium* prevalence with respect to habitat type (Ferraguti et al., 2018). This is a pioneering study in that it emphasizes the effect of deforestation on birds, parasites and mosquitoes at the same time in a small area of a tropical rainforest with the same general climate.

2. Materials and methods

2.1. Study area

The study took place in the Sithe Global-Sustainable Oils Cameroon (SG-SOC) concession (5°08′ to 5°20′N and 9°22′ to 9°24′E), located in the Talangaye rainforest, District of Nguti, Koupé-Manengouba Division, South West Region of Cameroon (Fig. 1). It is a forest corridor which lies between four protected areas (Korup National Park, Banyang-Mbo Wildlife Sanctuary, Rumpi Hills Wildlife Reserve and the Bakossi Mountains National Park) and has been undergoing large scale deforestation for the establishment of African palm oil trees (*Elaeis guineensis)* for palm oil production. It is made of tropical moist semi-deciduous and evergreen lowland forest, having an equatorial climate, exhibiting a single dry season (November to February) and one continuous rainy season (March to October), with an average annual rainfall of 3000 mm. The annual mean temperature and relative humidity are 30°c and 80%, respectively (Unites Councils and Cities of Cameroon, 2014. National office/Nguti Division. www.CVUC UCCC.com, Visited on the 13 May, 2018).

2.2. Data collection and laboratory analyses

Data was collected over 2 years in five Camps using mist netting (Dunn and Ralph, 2004). Only data from Camp 4, Camp 2, and Camp 6 representing each habitat type were used in this study. Habitats were characterized based on the main land cover type and the evidence of human activities (logging and palm oil tree settlements). Thereafter, three major categories of habitat were described and retained in the typology as follows: Camp 4, "pristine forest", defined as a forest with mature and tall trees showing no evidence of logging; it was characterized with large and tall trees of about 10 meter height, forming a continuous close canopy; Camp 2, "fragmented forest", defined as a forest patch which was slightly fragmented due to selective logging; it was characterized by the presence of tree stumps, felled trees, road openings and had a slightly open canopy. Data were collected in this Camp about two months after logging; then Camp 6, "young palm oil plantation", defined as a clear cut forest with establishment of young palm trees (less than 1 year old); it was characterized by the presence of dead wood, open ground, and grasses; it had a completely open canopy and was bordered by pristine forest that serves as a buffer. Data were collected in this Camp about three months after the establishment of the palm oil trees. In each Camp, three sites were determined based on where we could put up straight mist nets, and each site was monitored for 5 days. The three camps were in the same geographic area and have similar elevation (300–400 m). Pristine forest and fragmented forest were at about 10 km from each other, with a surface area of about 450 km^2 each. Distance between pristine forest and palm oil plantation was about 30 km, with a surface area of about 225 km^2 for plantation.

2.2.1. Mosquito sampling and identification

Mosquitoes were captured in the three habitat types using four sampling methods (sweep netting, Centers for Disease Control miniature light traps (CDC-LT), resting traps and bird baited net traps) in order to increase the probability of obtaining a high

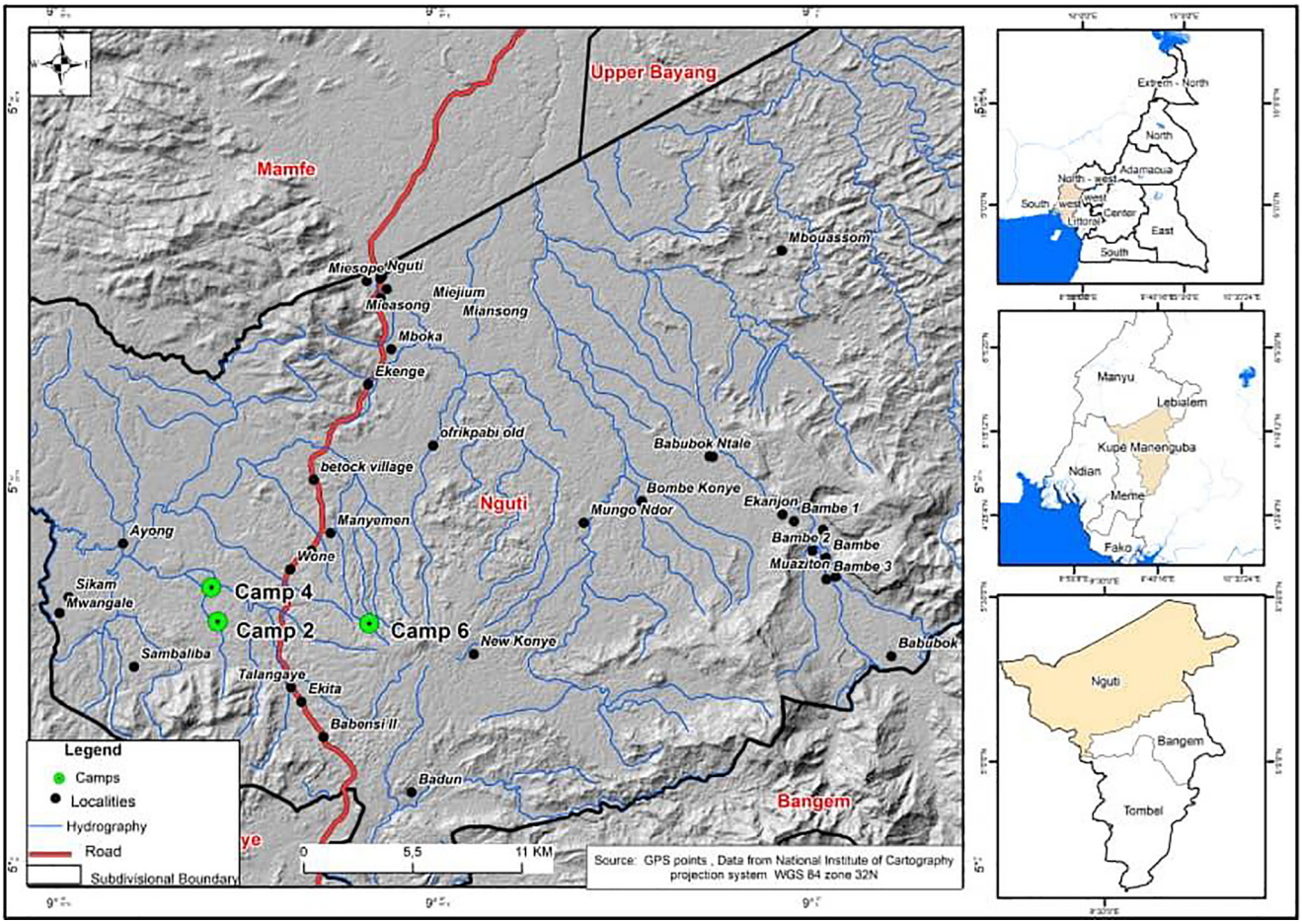

Fig. 1. Map of the study area in the South West Region, Cameroon.

diversity of mosquitoes in the different habitats (Carlson et al., 2015). Resting and bird baited net traps were constructed on the ground with tree branches of approximately 2 m in height and approximately 1 m × 1 m in breadth and depth. Bird baited traps were baited with chickens and pigeons held in cages within mosquito nets that were free of insecticide. The cages holding the birds were placed on a table or trestle made from tree branches. Both traps were set up for 24 h periods and checked every day by 4–6 people in the mornings and evenings. Sweep netting on the other hand was done daily, especially in the afternoon for 2–4 h by 4–6 people; unlike resting and net traps, which were fixed traps, sweep netting required more movement in the forest to look for mosquitoes resting on the vegetation. After capture, mosquitoes were knocked down with triethylamine (Sigma® St. Louis, MO, USA), sorted by gender, sex and date of collection, then morphologically identified to genus, subspecies, and when possible to species level with the aid of a stereomicroscope (X90) and morphological keys (Edwards, 1941; Service, 1990). Due to the focus on avian malaria, only female mosquito genera known to be ornithophilic and identified in previous studies as vectors of avian malaria were considered (this included genera *Aedes*, *Anopleles*, *Culex*, *Uranotaenia*,*Culiseta*, *Mansonia*, *Aedeomyia* and *Coquillettidia* (Valkiūnas, 2005; Ejiri et al., 2009; Njabo et al., 2009; Santiago-Alarcon et al., 2012; Okanga et al., 2013; Schmid et al., 2017; Abella-Medrano et al., 2018)). We did not collect hippoboscid flies nor ceratopogonid midges due to logistical issues and project aims.

2.2.2. Bird sampling

Birds were captured in the three habitat types at the same time as mosquitoes, using an average of 15 mist nets (12 m long, four shelves, 2.6 m high, 30 × 30 mm mesh) set up in parallel and/or perpendicular at each site and run for 7 h. Opened nets were checked every 15 min and all captured birds were identified using Borrow and Demey (2014); weighed using a Pesola scale of 100 and 1000 g; measured using a manual caliper with precision of 0.05; banded with numbered metal rings; sampled for blood and then released, after bleeding had stopped. Approximately 5–20 μL of blood were collected by venipuncture from the brachial vein of each captured bird and immediately stored in well-labeled cryotubes containing lysis buffer (10 mM Tris-Hcl, pH 8.0, 100 mM EDTA, 2% sodiumdodecylsulphate (SDS)), (Sehgal et al., 2001). Once in the laboratory, the samples were frozen at −20 °C until subsequent molecular analysis. Each species was then assigned to a dietary functional group based on information from Del Hoyo et al. (2016). Following their primary food choice, five dietary groups of birds were defined: carnivore, insectivore (insect eater), frugivore (fruit eater), granivore (seed eater), and nectarivore (nectar eater).

2.2.3. Parasite screening using microscopy

For each bird captured during field work, two thin blood films were prepared in the field using freshly drawn blood and blood smears were immediately air dried. In humid environments, a battery-operated fan was used to aid in drying of the blood smears. Smears were then fixed in absolute methanol for at least 1 min, air dried, packed into slide boxes and kept for subsequent staining in the laboratory. Once in the laboratory, all the blood films were stained for 1 h with Giemsa diluted in 1/10 with phosphate buffer and rinsed in tap water. After staining, blood films were air dried and at least 100 fields were examined at high magnification (×1000) under a light microscope (Olympus BX40 equipped with a canon micro photographic camera) using immersion oil.

2.2.4. Parasite screening using PCR

PCR was performed mainly on microscopy positive samples, for subsequent sequencing. However, samples that were negative by microscopy for *Plasmodium* and *Haemoproteus* parasites but positive for *Leucocytozoon* were also screened using PCR, for further confirmation. DNA was extracted from the whole blood samples using the DNeasy kit (Qiagen, Valencia, California, USA) following the manufacturer's guidelines. The presence/quality of DNA extracted was verified by doing a PCR of 10% extract selected randomly among the samples using universal primers BDNF3 and BDNF5 (Sehgal and Lovette, 2003), to amplify all DNA present in the blood before using specific primers for parasites. Further complementary PCRs were then run to amplify a fragment of the mitochondrial DNA cytochrome b gene of the parasites, but differed in primer design and the number of cycles included in the PCRs. We used the nested PCR approach of Hellgren et al. (2004), with an initial amplification of a 617 bp long fragment common to the three genera of haemosporidian parasites (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*), using primers HaemNF (5′-CATATATTAAGAG AATTATGGAG3′) and HaemNR (3′AGAGGTGTAGCATATATCTATCT AC5′). A subsequent PCR using a combination of primers HaemF (5′ATGGTGCTTTCGATATATGCATG-3′) and HaemR2 (3′-GCAT TATCTTGGATGTGATAATGGT-5′) was performed to detect *Haemoproteus*/*Plasmodium* spp.

The initial PCR was performed in a 25 μL volume, included in puReTag Ready-To-Go ™ PCR Beads (Illustra™, GE Healthcare, UK). The bead was made up of dNTP, Tris-HCL, KCL, MgCL2, reaction buffer and puReTaq DNA polymerase. Once mixed, the solution was put in well-labelled PCR tubes and loaded in a PCR machine (BioRad T100™ thermal cycler) for amplification. The amplification protocol started with 3 min at 94 °C (initial heating); followed by 20 cycles at 94 °C for 30 s (denaturation); 50 °C for 30 s (annealing); 72 °C for 45 s (elongation); and ended at 72 °C for 10 min as a final elongation or termination step. For the subsequent PCR, we used 2 μL of the initial PCR products as template. This PCR was performed using the same reagents as described above (except primers), but ran for 35 cycles. Positive controls (samples from birds with proven *Plasmodium*/*Haemoproteus* infections) and negative controls (PCR grade water) were used to verify the result of each PCR run. Positive and negative infections were evaluated by running 2 μL of the final PCR products on 2% agarose gel stained with ethidium bromide and 1 μL of loading dye (LGC Biotechnology®, UK). To determine the size of the fragment, 5 μL of a 100 bp DNA ladder (AMRESCO®) were used, the gel was run for 1 h at 150 V and 108 A and bands were visualized under ultraviolet light using a transilluminator (BIORAD Gel Doc™).

2.2.5. Ethics

The study was approved by the Animal Experimentation Ethics Committee of the University of Buea, Cameroon. Protocols used have been reviewed and accepted by United States Agency for International Development (USAID) through Partnerships for Enhanced Engagement in Research (PEER, project 4-360). A risk assessment was performed before each field trip and appropriate equipment provided for each participant.

2.3. Statistical analyses

All samples positive by microscopy and/or by PCR were considered in the analyses. We did not separate microcopy results from PCR results. We used generalized linear models (GLMs) of binary observations to model the infection status associated with each parasite. More specifically, we assumed

$$Y_i \sim \text{Bernoulli}(\psi_i)$$

where Y_i denotes a random variable for the infection status of the ith bird (Y_i = 1 if the ith bird is infected; otherwise, Y_i = 0). The parameter ψ_i is the probability that the $_i$th bird is infected with the parasite (that is, $\psi_i = \Pr(Y_i = 1)$). The value of this parameter,

commonly referred to as the "prevalence" of the parasite in the host population, is unknown and must be estimated from the binary observations in the sample.

To assess whether the prevalence of parasites in a specific group of birds (we considered groups that differed by species, by taxonomic family, and by dietary guilds) differed among habitats, we used likelihood ratio tests by comparing two nested models: one where prevalences were assumed to differ among groups (the null model), and another where prevalences were assumed to differ among groups and habitats. The latter model included parameters for group-habitat interactions so that the effects of habitat could differ among groups. We also used likelihood ratio tests to assess whether the prevalence of parasites in an individual group of birds differed among habitats. In these tests, models with and without habitat effects were compared for each group of birds.

The effect of habitat on the overall prevalence of *Plasmodium* and *Haemoproteus* was assessed using GLMs with prevalence (ran with a binomial error structure) as the response variable and habitat types as explanatory variables. Mosquito abundance was correlated to *Plasmodium* prevalence in each habitat using Spearman's rank correlation tests. All the analyses were performed in R software (R Core Team, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org), and the results were considered significant for $P < 0.05$.

3. Results

3.1. Habitat affects the overall prevalence of Plasmodium and Haemoproteus

Overall, we screened 845 bird individuals of 85 species and 27 families, sampled in three habitat types (pristine forest, fragmented forest, and young palm oil plantation). *Plasmodium* infections were detected in 136 individuals (16.09%) and *Haemoproteus* infections in 98 individuals (11.60%). The prevalence of *Plasmodium* infection varied significantly among habitat types and was associated with young palm oil plantation (GLM: −0.83 ± 0.29, $P = 0.004$), while the prevalence of *Haemoproteus* infection did not vary significantly among habitat types (Fig. 2). The highest *Plasmodium* prevalence (21.52%) was recorded in pristine forest while young palm oil plantation had the lowest *Plasmodium* prevalence (7.93%). For *Haemoproteus* infection, the highest prevalence was also recorded in pristine forest (12.34%), while fragmented forest registered the lowest *Haemoproteus* prevalence (10.60%). The trends for *Plasmodium* and *Haemoproteus* infections among habitat types differed. *Plasmodium* infection decreased with forest fragmentation and more with forest loss through the establishment of the young palm oil plantation while *Haemoproteus* infection decreased with forest fragmentation but slightly increased after the establishment of the young palm oil plantation.

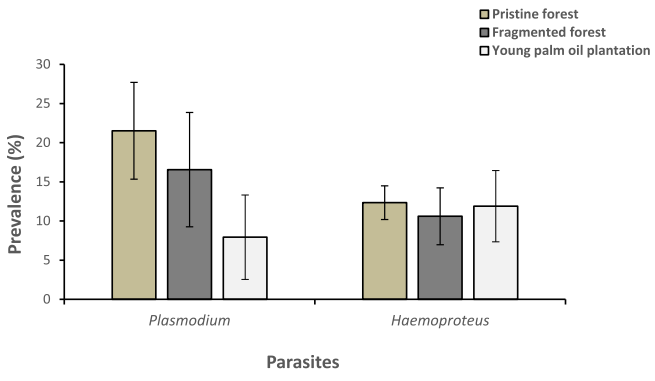

Fig. 2. Prevalence of *Plasmodium* and *Haemoproteus* infections among habitat types. Bars represent standard errors.

3.2. Habitat affects the prevalence of Plasmodium and Haemoproteus in bird groups

Of the 85 avian species sampled for *Plasmodium* and *Haemoproteus* infections, 49 (56.98%) were infected, while 37 (43.02%) were not infected. Of the 49 species that showed detectable infection, 15 were infected only with *Plasmodium*, 17 were infected only with *Haemoproteus* and 17 had both *Plasmodium* and *Haemoproteus* infections (Supplementary Table S1). Overall, the Brown-chested Alethe (*Chamaetylas poliocephala*) had the highest *Plasmodium* prevalence (16/25 (64%)), while the Olive sunbird (*Cyanomitra olivacea*) had the highest *Haemoproteus* prevalence (21/98 (21.43%)). The prevalence of *Plasmodium* and *Haemoproteus* in most bird species did not differ significantly among habitats ($P = 0.80$ for *Plasmodium* and $P = 0.64$ for *Haemoproteus*). However, when analyzing each bird species individually (Fig. 3), *Plasmodium* prevalence in the Brown-chested Alethe (*C. poliocephala*) was significantly higher in fragmented forest than in pristine forest ($P = 0.005$) while *Haemoproteus* prevalence in the Olive sunbird (*C. olivacea*) was significantly higher in fragmented forest and in the young palm plantation than in pristine forest ($P = 0.005$).

The most prevalent *Plasmodium* family was Nectariniidae (28/144 (24.56%)), while the family Monarchidae appears to have the highest *Haemoproteus* prevalence (7/17 (41.18%)). Estimates of *Plasmodium* and *Haemoproteus* prevalence in bird families and habitats (Fig. 4) revealed that *Plasmodium* prevalence in bird families did not vary significantly among habitats ($P = 0.46$), while *Haemoproteus* prevalence varied significantly among habitats ($P = 0.04$). Analyses of each family separately showed that *Plasmodium* prevalence in the family Pycnonotidae was significantly higher in pristine forest than in fragmented forests ($P = 0.024$). However, the differences in *Haemoproteus* prevalence were evident in two families (Nectariniidae and Pycnonotidae). In the family Nectariniidae, *Haemoproteus* prevalence was significantly higher in fragmented forest ($P = 0.002$) than in pristine forest while for the family Pycnonotidae, this parasite was absent in the young palm oil plantation but infection rates significantly increased in fragmented and pristine forests ($P = 0.03$).

The number of carnivorous birds was too low to permit analyses. The prevalence of *Plasmodium* and *Haemoproteus* in the other feeding groups across habitat types is summarized in Fig. 5. In total, 501 insectivores, 180 frugivores, 54 granivores, and 109 nectarivores were recorded and examined. Nectarivorous birds had the highest *Plasmodium* prevalence (28/109 (25.69%) while granivores had the highest *Haemoproteus* prevalence (15/54 (27.78%). Fig. 5 summarizes the estimated prevalence of *Plasmodium* and *Haemoproteus* in bird feeding guilds and habitats. Overall, the prevalence of *Plasmodium* in feeding guilds differed significantly among habitats ($P < 0.001$). However, the differences in *Plasmodium* prevalence were evident in only two guilds (frugivores and insectivores). In frugivores, *Plasmodium* prevalence was significantly higher in pristine forest than in fragmented forest or in the young palm oil plantation ($P = 0.049$). In insectivores, *Plasmodium* prevalence was significantly lower in the young palm oil plantation than in fragmented forest or pristine forest ($P < 0.001$). The prevalence of *Haemoproteus* in feeding guilds differed significantly among habitats ($P < 0.001$). However, the differences in prevalence were evident in only two guilds (frugivores and nectarivores). In frugivores, *Haemoproteus* prevalence was significantly lower in the young palm oil plantation than in fragmented forest or pristine forest ($P = 0.039$). In nectarivores, *Haemoproteus* prevalence was

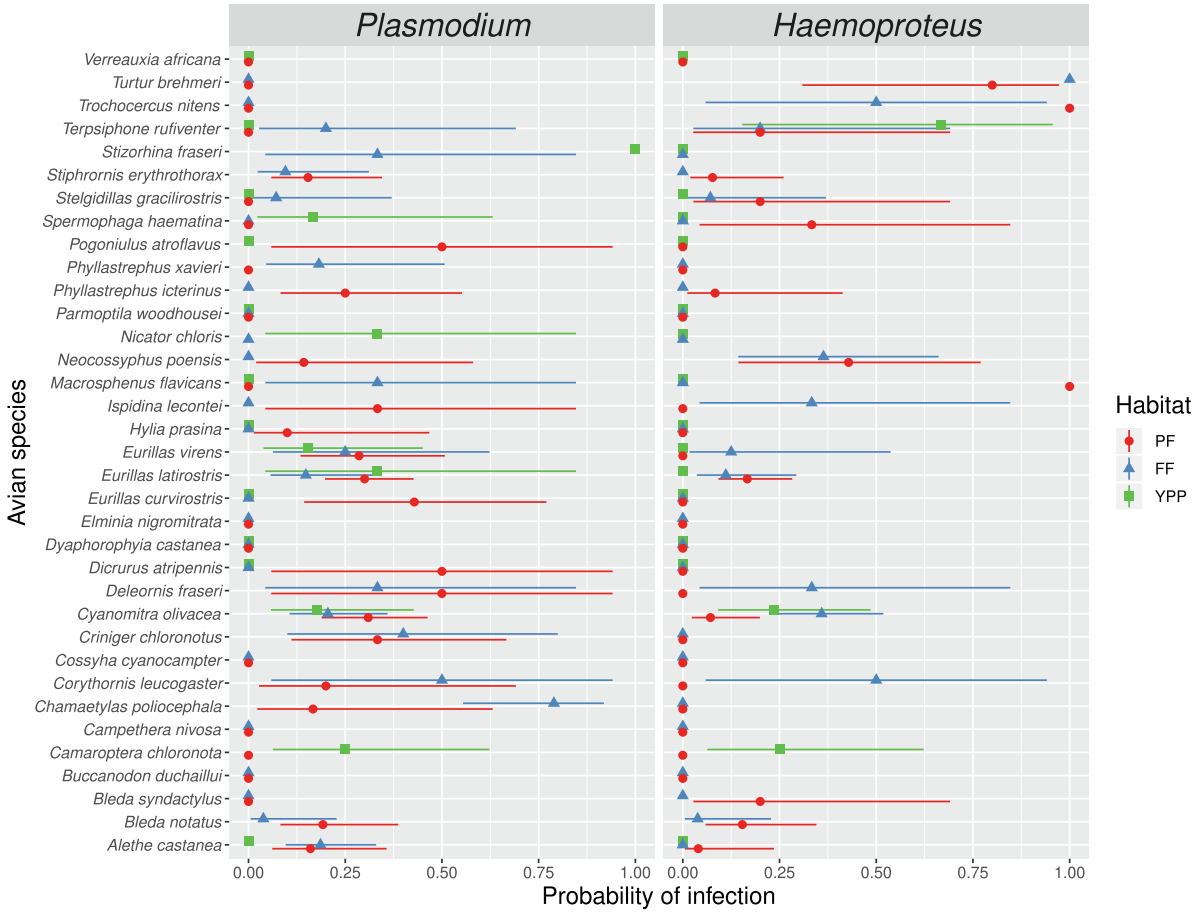

Fig. 3. Estimates of *Plasmodium* and *Haemoproteus* prevalence in different bird species and habitats. PF, pristine forest; FF, fragmented forest; YPP, young palm oil plantation. Error bars indicate 95% confidence intervals.

significantly higher in fragmented forest than in pristine forest or in the young palm plantations ($P = 0.003$).

3.3. *Plasmodium* prevalence correlates with mosquito abundance

Overall, a total of 1,194 female mosquitoes belonging to 65 species and four genera were collected in the three habitat types. Three hundred and eighty-five individuals (32.24%) of 27 species were recorded in pristine forest, 687 individuals (57.54%) of 43 species were recorded in fragmented forest and 122 individuals (10.22%) of 20 species were recorded in the young palm oil plantation (Supplementary Table S2). The abundance of mosquitoes varied significantly among habitat types ($P < 0.001$). A Spearman rank correlation test showed that *Plasmodium* prevalence significantly and positively correlated with mosquito abundance in fragmented forest ($r = 0.36$, $P = 0.02$). This relationship was also evident in species, in families, and in feeding guilds of birds. The avian malaria mosquito genera found in our study were: *Culex* (81.24%), followed by *Aedes* (10.13%), *Uranotaenia* (6.53%) and *Anopheles* (2.1%). All were encountered in the three habitat types, but very low abundance of species from *Uranotaenia* and *Anopheles* genera were recorded in the young palm oil plantation.

4. Discussion

Here we investigated the relationship between avian haemosporidian infections, bird feeding behavior and mosquito abundance in response to habitat changes. To our knowledge, it is the first time that this kind of comprehensive study has been done in sub-Saharan Africa. We found that *Plasmodium* and *Haemoproteus* prevalence decreased after habitat fragmentation but after the establishment of the young palm oil plantation, *Haemoproteus* prevalence slightly increased while *Plasmodium* still decreased. When studying habitat effects on parasites, it is most likely the vector stage that will most greatly influence the prevalence and parasitemia (Sehgal, 2015). In addition, methods and timing of bird capture can influence the observed prevalence and parasitemia (Valkiūnas, 2005). Some evidence also suggested that the prevalence of parasites in a bird community is affected by the differences in host species composition, as well as geography and vector ecology (Scordato and Kardish, 2014). In our study, sampling methods, timing (seasonality) as well as geography could not be predictors of the observed differences in prevalence. In fact, the same sampling methods and effort were applied in all three habitat types and these habitats were within the same small geographic area. By comparing habitats on a reduced spatial scale, we eliminated many of the confounding environmental variables (such as rainfall, temperature) that could affect parasite transmission. Studies of the seasonality in our study area were done and showed no significant variation in avian haemosporidian prevalence among seasons (unpublished data). Vegetation structure could better explain the observed differences in the prevalence of *Plasmodium* and *Haemoproteus* parasites among habitat types. Pristine forest and fragmented forest had almost the same vegetation structure, while the young palm oil plantation was different, although surrounded

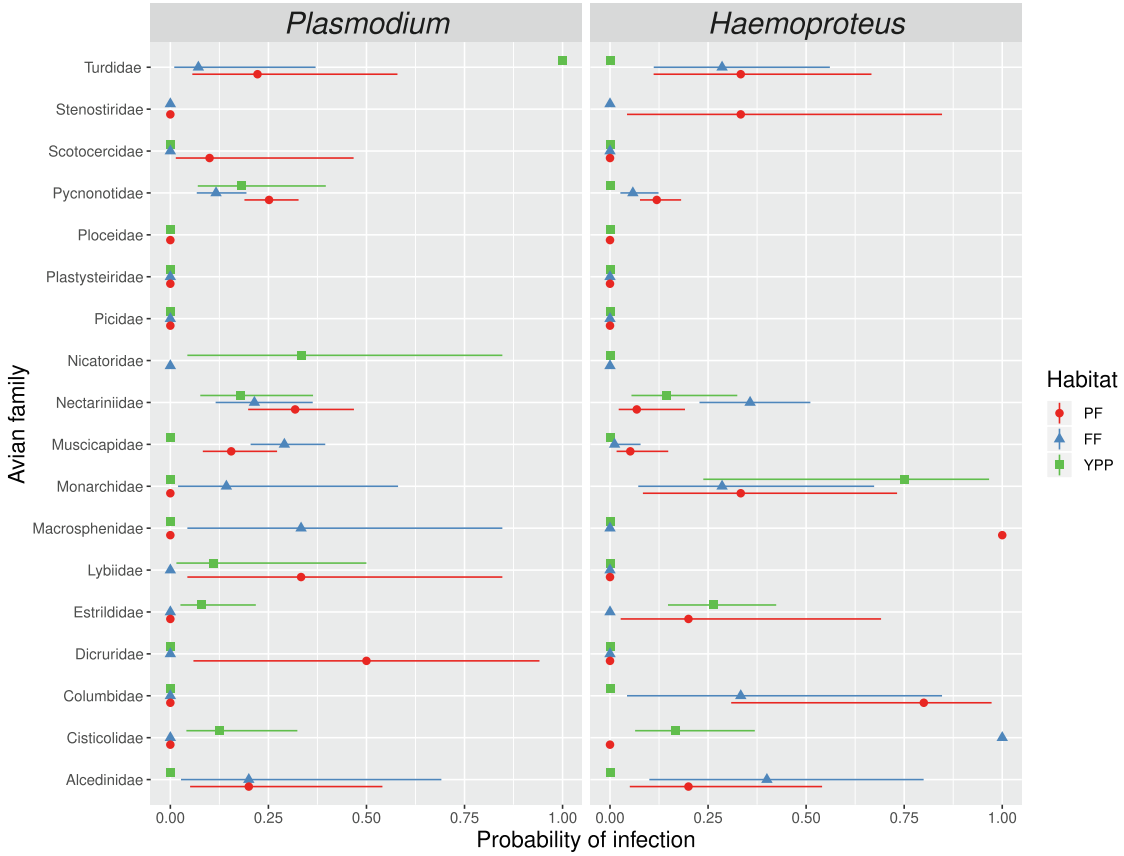

Fig. 4. Estimates of *Plasmodium* and *Haemoproteus* prevalence in different bird families and habitats. PF, pristine forest; FF, fragmented forest; YPP, young palm oil plantation. Error bars indicate 95% confidence intervals.

by pristine forest, which served as a buffer. Vegetation is crucial in providing cover for dipteran breeding sites and for forest mosquitoes, mature areas may offer better vector breeding grounds than disturbed areas (Yasouki, 2007). The prevalence of parasites in an area is not only associated with the abundance of vectors but also vectorial capacity, which should be taken into account.

On the other hand, young palm oil plantations may result in the introduction of novel vectors and increase competition among vectors with the decline in the abundance of native competent vectors. For example, Vittor et al. (2006) demonstrated that the mosquito *Anopheles darlingi*, the primary vector for human malaria in the Amazon, was found to be absent in intact habitats and present in altered sites, with a biting rate increasing by almost 300%. In addition, other studies have reported feeding shifts by mosquitoes from birds to humans when the numbers of preferential hosts were reduced (Kilpatrick et al., 2006). The decreased prevalence of infection in the young palm oil plantation could also be explained by changes in the feeding habits of insect vectors, especially vectors that were relatively non-selective in their food preferences. Vectors could change from primarily zoophilic to primarily anthropophilic with ecological disruption, resulting in a decrease in parasite prevalence in the original hosts (Chasar et al., 2009). Our results are similar to that of Bonneaud et al. (2009) who reported a significantly greater avian *Plasmodium* prevalence in undisturbed mature forest compared with disturbed forest in Cameroon. Similar results were also found with *Haemoproteus* infection in northern Australia (Laurance et al., 2013). In contrast to our findings, Belo et al. (2011) reported no significant difference in the prevalence of *Plasmodium* and *Haemoproteus* in three habitats in Brazil. Rivero de Aguilar et al. (2018) also reported an absence of relationship between forest fragmentation and bird infection status. However, taken as a whole, their findings indicate little effect of fragment characteristics per se on avian haemosporidian infections, although additional sampling would have offered more power to detect potential relationships.

When all the individuals were considered in the same model, the prevalence of *Plasmodium* and *Haemoproteus* did not vary significantly among species, but *Haemoproteus* infection significantly varied among bird families. The highest *Plasmodium* prevalence was found in the Brown-chested Alethe (*Chamaetylas poliocephala*), while the highest *Haemoproteus* prevalence was recorded in the Olive sunbird (*Cyanomitra olivacea*), with a significant increase in fragmented forest. This result is similar to that of Chasar et al. (2009), who reported that in the Olive sunbird (*C. olivacea*) a higher prevalence of haemosporidian infections occurred in disturbed habitat compared with undisturbed habitat in Cameroon. The effects of haemosporidian parasites on hosts have been shown to vary in relation to the species of the host (Bonneaud et al., 2006; Loiseau et al., 2008). Differences in prevalence between bird species and bird families might be due to the trade-offs that occur in their evolutionary strategy during immune system investment (Norris and Evans, 2000; Tomas et al., 2007). This evolutionary strategy is thought to be altered by habitat degradation/conversion. Alternatively, these two species (*C. poliocephala* and *C. olivacea*) might differ in their attractiveness to vectors compared with other bird species. Indeed, feeding specialization of vectors or behaviour of the hosts might also contribute to this result. Our findings also suggest that there was large interspecific variation in parasite prevalence within the avian communities. This result is similar to that of Sebaio et al. (2012) who studied the blood

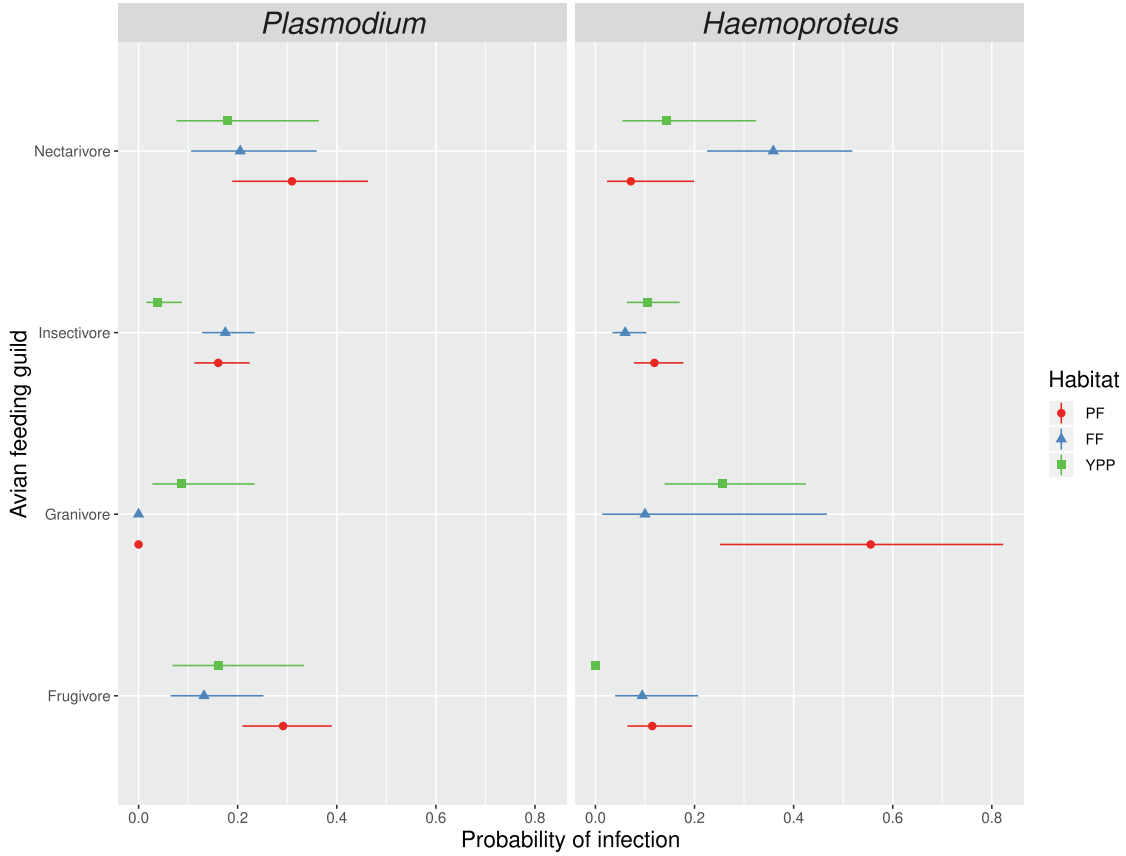

Fig. 5. Estimates of *Plasmodium* and *Haemoproteus* prevalence in different bird feeding guilds and habitats. PF, pristine forest; FF, fragmented forest; YPP, young palm oil plantation. Error bars indicate 95% confidence intervals.

parasites in passerine birds from the Brazilian Atlantic Forest. There may be species-specific differences in immune responses and susceptibility to infections among host species and families, or infection might be connected to certain ecological or life history traits of the species. Among the blood parasites, *Haemoproteus* are generally thought to be more host-specific than *Plasmodium* (Beadell et al., 2009; Zamora-Vilchis et al., 2012). The observed variation among species and families was not associated with the abundance of species, since the most abundant species was the Yellow-whiskered greenbul (*Eurillas latirostris*) and the most individually rich family was the family Pycnonotidae. The considerable differences in parasite prevalence found at species and family levels illustrated the complexity of the study, and set a high demand on the sample sizes needed to draw conclusions about general trends.

The proportion of individuals infected by *Plasmodium* and *Haemoproteus* varied significantly among feeding groups. Our results are in accordance with that of Fandrem (2015) who studied the effects of selective logging on avian blood parasites in a Bornean rainforest (Fandrem, M. 2015. Bird-parasite dynamics in a Bornean rainforest: the effects of selective logging and host characteristics. Masters thesis, Norwegian University of Life Sciences, 88), but is opposite to that of Fecchio et al. (2017) who reported an absence of relationship between ecological traits (including diet) of the birds and the prevalence of *Plasmodium* parasites in southeastern Amazonia. Surprisingly, nectarivores and granivores had the highest proportion of *Plasmodium* and *Haemoproteus* infections, respectively. It is possible that these two groups of birds encounter unusual vectors in our study area and since their immune system is not yet used to those vectors, they are infected more often than, for example, insectivorous birds, which are frequently in contact with vectors since they feed on them. The highest prevalence observed in these two bird feeding groups is more associated with pristine forest. This result is similar to that of Latta and Ricklefs (2010) who studied bird assemblages in four habitats at study sites near Cabo Rojo and in the Sierra de Bahoruco, Dominican Republic. Opposite to our findings, Laurence et al. (2013) found *Haemoproteus* infection to be significantly higher in insectivorous birds in northern Australia. Similarly, the study of Gonzalez et al. (2014) revealed that omnivorous birds had higher *Plasmodium* prevalence in Colombia. The effect of forest disturbance on the transmission of avian haemosporidian parasites in different bird feeding groups is still understudied. Habitat fragmentation seems to favor an increase in the prevalence of *Plasmodium* in insectivores, while it favors an increase in the prevalence of *Haemoproteus* in nectarivores. However, the establishment of the palm oil plantation favors an increase in the prevalence of *Plasmodium* in granivores, while it favors an increase in prevalence of *Haemoproteus* in nectarivores. The present study offers insight into how habitat changes alter the transmission of haemosporidians according to the feeding behavior of bird species. Many insectivores and granivores are considered opportunistic species and unlikely to be impacted by logging (Meijaard et al., 2005), and often increase in numbers. A recent study on the response of bird feeding groups to habitat changes in our study area also reported an increase in the abundance and species richness of granivorous and insectivorous birds after habitat fragmentation and the establishment of palm oil plantations (Tchoumbou et al., unpublished). More hosts and host species becoming available to vectors will probably increase the prevalence of infections.

In the present study, the abundance of mosquitoes varied significantly among habitat types. The highest abundance was registered in fragmented forest and the lowest abundance was in the young palm oil plantation. This result is similar to that of Abella-Medrano et al. (2018) who found abundances of mosquito communities to vary greatly among five different land use types within a neotropical montane, with higher values in the forests and lower values in the coffee plantation. In addition, van Hoesel et al. (2019) reported that higher forest management intensity decreased vector abundance. The fragmented forest in our study was defined as a forest somewhat fragmented due to selective logging. Such disturbance possibly allowed sunlight penetration and accelerated mosquito development and survival (Kilpatrick et al., 2006; Santiago-Alarcon et al., 2013; Zahouli et al., 2016; Tangena et al., 2016). Many mosquito species in forests live in the upper canopy of large trees. Removal of these trees may force these canopy mosquitoes downwards to ground levels and thus may also contribute to higher abundance in the understory in fragmented forests. These factors in combination with the great number of larval biotopes that are still available to be exploited by different species may also induce an increase in the abundance of mosquito transmitted parasites (Nikookar et al., 2015). This was not the case in the young palm oil plantation which was characterised by the complete removal of natural vegetation and losses of natural breeding sites, which might lead to the reduction and disappearance of larval populations of certain mosquito species (Barros et al., 2011). The young palm oil plantation instead favoured the invasion of anthropophilic mosquitoes such as *Aedes albopictus* and *Aedes aegypti*, which were only found after deforestation (unpublished data).

On the other hand, *Plasmodium* prevalence significantly and positively correlated with the abundance of mosquitoes in fragmented forest. This result is in accordance with that of Ferraguti et al. (2018) who reported that avian *Plasmodium* prevalence was explained by vector-related variables. However, these researchers did not thoroughly investigate the effect of habitat type. Interactions between mosquito vectors and *Plasmodium* parasites in a changing environment are not yet well documented. Prevalence, diversity and distribution of avian parasites might be constrained by the definitive host (Culicidae) in the bird-parasite-mosquito system. The observed correlation in our study can mainly be explained by the highest number of ornithophilic and avian malaria vectors registered in fragmented forest. The abundance of avian malaria vectors in an area might affect avian parasite prevalence, since they are responsible for the transmission of *Plasmodium* spp. in hosts (Manguin and Boëte, 2011; Simpson et al., 2012; Takken and Verhulst, 2013; Roche et al., 2013). Mosquitoes of the genera *Culex*, *Aedes*, *Anopheles* and *Uranotaenia* which have been implicated in the transmission of avian *Plasmodium* spp. (Valkiūnas, 2005; Ejiri et al., 2009; Njabo et al., 2009; Okanga et al., 2013; Schmid et al., 2017; Abella-Medrano et al., 2018), were found to be abundant in the fragmented forest compared with other habitats (Supplementary Table S2). Interestingly, mosquitoes from the *Culex* genus were by far the most abundant in fragmented forest (representing 81.24% of the total collections and 77.44% of the total mosquitoes collected in the fragmented forest). Many species of *Culex* have been described as vectors of avian malaria parasites (Santiago-Alarcon et al., 2012). More specifically, species such as *Culex. neavei*, *Culex. perfidiosus*, *Cules. poicilipes* and *Culex. guiarti* have been described as vectors of avian *Plasmodium* in Cameroon (Njabo et al., 2011). Ferreira et al. (2016), in their study in Brazil, also found a higher abundance of putative vectors of avian malaria (including *Culex* spp.) in recently disturbed habitats (5–20 years old abandoned cattle grazing) than in very old (50 years old abandoned cattlegrazing) disturbed habitats (re-established forest with tall deciduous trees forming a closed canopy at 18–20 m from ground level).

The mosquito species richness was also found to be higher in fragmented forest compared with the other habitats. Among those species, some absent in pristine forest were found to be present in fragmented forest (Supplementary Table S2). The exposure of birds to new mosquito species might increase their vulnerability, since their immune system is not yet used to new strains of *Plasmodium* that could be transmitted by those new mosquito species. The match-ups between avian hosts and mosquito vectors between habitat types could also explain the observed correlation in fragmented forest. The mosquito community in fragmented forest might have had a higher impact on parasite prevalence due to the low specificity of some of the caught mosquitoes that transmit avian *Plasmodium* (Kimura et al., 2010). More studies are needed to identify the characteristics of the land use which affect the mosquito community and contribute to an explanation of changes in parasite prevalence. Very little is yet known about the biology (including feeding behavior and vector role) of forest dwellers, as in the case of Talangaye mosquitoes. Future experimental studies to determine whether these mosquitoes harbour the infective sporozoites of *Plasmodium*, for which host bird species could be the blood meal source, are also necessary to demonstrate vectorial capacity.

Our study provides additional information on the implications of some host and mosquito determinants in the transmission of avian *Plasmodium* and *Haemoproteus* infections following two major phenomena (habitat fragmentation through selective logging, and habitat loss through the establishment of a palm oil plantation). The prevalence of *Plasmodium* infection decreased after habitat fragmentation and habitat loss while *Haemoproteus* prevalence decreased with habitat fragmentation but slightly increased following habitat loss. Insectivores, nectarivores and granivores were more susceptible to avian *Plasmodium* and *Haemoproteus* infections following deforestation. Analyses of the relationship between mosquito abundance and prevalence of *Plasmodium* revealed a significant and positive correlation in fragmented forest. Nonetheless, our study is still limited in understanding the complex interactions between birds, haemosporidian parasites and vectors in a changing environment. Environmental context plays a determinant role in regulating the prevalence and diversity of pathogens, thus analyses of vast data sets integrating all of the players affecting the dynamics of vector-borne pathogens is recommended.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2019.10.006.

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