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# Mosquito communities across land-use gradients in Amazonia and the Cerrado

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Campo Grande
Maio, 2021

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# Comunidades de mosquitos em gradientes de uso da terra na Amazônia e no Cerrado

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Tese apresentada como requisito para a obtenção do título de **Doutor em Ecologia**, pelo Programa de Pós Graduação em Ecologia e Conservação, Universidade Federal de Mato Grosso do Sul.

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*Ao meu co-orientador Wanderli Pedro Tadei (in memoriam), que deixou um legado em forma de conhecimento sobre Malária e Dengue na Amazônia, minha eterna gratidão por tudo.*

# Agradecimentos

À oportunidade de ter crescido em contato com a floresta Amazônica, onde auxiliou diretamente na minha admiração por ela, suas interações e assim buscando melhor entendê-la utilizando a pesquisa como ferramenta;

À minha querida mãe Rozana, avó Eimar, irmãos e família por me incentivarem e acima de tudo orgulho do meu trajeto percorrido;

Ao meu orientador Dr. Fabio Roque por ter me acolhido na sua equipe de trabalho em Campo Grande e ter abraçado a ideia do projeto com mosquitos vetores, onde pude aprender novas perspectivas na Ecologia;

Ao meu co-orientador Dr. Wanderli Pedro Tadei que nos deixou recentemente, meu total agradecimento e gratidão pela oportunidade de conhecer e me encantar pela área da Entomologia Médica, onde pude crescer e amadurecer todo o tempo em que estive no Laboratório de Malária e Dengue do INPA;

À colaboradora Dra. Hillândia Brandão da Cunha por ter me acolhido na sua equipe de pesquisa “Limnologia” desde início do Pibic em 2007 no INPA e confiado no meu trabalho até hoje;

Aos Mestres e Doutores que ajudaram direta e indiretamente com conselhos, parcerias, material de estudo, atenção e uma grande amizade “Dra. Rosemary Roque, Dr. João Zequi, Dr. Fernando Carvalho, Dr. Francisco Valente-Neto, Dr. Robert Hughes, Me. Philip Teles”;

À toda equipe do Grupo de Pesquisa Coexistência e Decisão, ao Laboratório de Ecologia da UFMS, Laboratório de Química Ambiental do INPA e Laboratório de Malária e Dengue do INPA pelo auxílio nas coletas, análises, identificações, dúvidas, execução e principalmente pela amizade que foi criada e mantida;

Ao Prof. Erich Fischer por ter disponibilizado a logística dos seus projetos em andamento para a execução do meu terceiro capítulo no Planalto da Bodoquena, sendo de grande importância para o a finalização desta Tese. Agradeço também aos técnicos, alunos e colegas que me ajudaram nessa etapa do trabalho, em especial no campo: Carol, André, Adriana, Audrey, Sr. Almir, Maxwell e Fábio Henrique.

Ao corpo docente e funcionários do curso de PPG em Ecologia e Conservação e PPG em Biologia Animal da UFMS, pelo suporte ao longo do doutorado e por ter possibilitado o contato com a natureza dessa região única, em especial a vivência no EcoPan2017;

As pessoas maravilhosas que somaram na minha vida e que foram meu alicerce durante os quatros anos e meio em Campo Grande: Luis Otávio, Camyle, Eric, Paula, Karol, Douglas, Adriana, Maxwell, Mateus, Nathália, Murillo, Maiara, Vivian, Tai, Thais, Larissa, Philip, Nathalie, Matheus, Mariana, Samara, Ana Cláudia, Caroline, Dona Luciana, Letícia, Carol, Dona Marizete, e muitos e muitos outros;

As grandes amizades que sempre são cultivadas presentes na minha vida: Talita, Augusto, Taís, Janaína, Rebeca, André, Gilberto, Eduardo, Tides, David, Camila, Lívia, estas com suas peculiaridades e alegrias em minha vida;

À Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT) pelo suporte e bolsa concedida na Chamada FUNDECT n° 03/2017 – Doutorado em Mato Grosso do Sule, à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001” pela bolsa de estudos concedida, à Universidade Federal de Mato Grosso do Sul (UFMS) pelo suporte logístico e financeiro, e oportunidade de cursar o doutorado, e aos colaboradores diretos e indiretos que contribuíram para a realização da Tese;

À banca avaliadora da Tese por ter eceito o convite e pela contribuição para a melhoria do trabalho final;

À todos que contribuíram de alguma forma no meu estabelecimento em Campo Grande e no desenvolvimento da Tese, meu muito obrigado.

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# General abstract

Changes from natural landscapes to anthropogenic landscapes have caused negative effects on species biodiversity, imbalance between vectors, pathogens and their hosts, leading to a significant increase in the number of some species of mosquitoes, mainly anthropophilic. Our knowledge is still limited to identifying the levels of transformation that generate critical changes in the ecological system, whether the mosquito communities change gradually or whether the changes are drastic and sudden. Perhaps the most important of these predictors is the total amount of native vegetation remaining. In addition, environmental changes play an important role in the emergence of new habitats for various species of vector mosquitoes. Most studies on mosquitoes detect the effect of forest cover on mosquito biodiversity, in addition, environmental variables are being related to the presence of species in natural and altered areas (e.g. rainfall, humidity, temperature, water quality of larval habitat). In this study, in the three chapters we seek to: (1) characterize the larval habitat of *Anopheles* and identify limnological variables associated with the presence of species; (2) evaluate how the loss of forest can directly and indirectly affect anopheline assemblies in both seasons; (3) to identify the effect of loss of native vegetation along a gradient of forest cover on the richness and abundance of vector and non-vector mosquitoes, and to identify thresholds for each species. In the first and second chapter, collections of immatures were carried out in several larval habitats of *Anopheles* in the peri-urban area of the city of Manaus - AM, and the third chapter we collected the Culicidae community along a gradient of forest cover in an area of Cerrado in the Bodoquena Plateau - MS. We used canonical correlation analysis to verify the relationship between limnological variables and the anopheline larval presence. In addition, we use structural equation modeling to investigate the effects of forest cover on the water quality of habitats and on the larval anopheline assemblage in different seasons. For adult mosquitoes, we selected linear and non-linear models to assess the response of mosquito richness and abundance to forest loss. In summary, we found strong evidence that anopheline larvae are affected by the water quality of the habitat and that limnological variables play an important role in the presence of certain species. In addition, the forest plays an important role in these habitats, directly and indirectly affecting the anopheline assemblage in habitats in the dry and rainy season. On the other hand, in the terrestrial environment, mosquitoes are influenced by forest cover, with an increase in total abundance. However, vector species are benefited by the decrease in forest cover and the reverse is observed for non-vector species.

# Resumo geral

Mudanças de paisagens naturais para paisagens antropogênicas têm causado efeitos negativos na biodiversidade de espécies, desequilíbrio entre os vetores, patógenos e seus hospedeiros, levando a um aumento significativo no número de algumas espécies de mosquitos, principalmente antropófilas. Nosso conhecimento ainda é limitado para identificar os níveis de transformação que geram mudanças críticas no sistema ecológico, se as comunidades de mosquitos mudam gradualmente ou se as mudanças são drásticas e repentinas. Talvez o mais importante desses preditores seja a quantidade total de vegetação nativa remanescente. Além disso, as modificações ambientais exercem papel importante para o surgimento de novos hábitats para diversas espécies de mosquitos vetores. A maioria dos estudos sobre mosquitos detectam o efeito da cobertura florestal na biodiversidade de mosquitos, além disso, as variáveis ambientais estão sendo relacionadas com a presença de espécies em áreas naturais e alteradas (exemplo: chuva, umidade, temperatura, qualidade da água dos criadouros). Neste estudo, nos três capítulos buscamos: (1) caracterizar o habitat larval de anofelinos e identificar variáveis limnológicas associadas com a presença de espécies; (2) avaliar como a perda de floresta pode afetar direta e indiretamente as assembléias de anofelinos nas duas estações (seco e chuvoso); (3) identificar o efeito da perda de vegetação nativa ao longo de um gradiente de cobertura florestal sobre a riqueza e abundância de mosquitos vetores e não vetores, e identificar limiares para cada espécie. No primeiro e segundo capítulo foram relizadas coletas de imatudos em diversos hábitats larvais de anofelinos na área peri-urbana da cidade de Manaus – AM, e o terceiro capítulo foram coletados mosquitos adultos ao longo de um gradiente de cobertura florestal em uma área de Cerrado no Planalto da Bodoquena – MS. Utilizamos a análise de correlação canônica para verificar a relação das variáveis limnológicas com a presença larval de anofelinos, além disso usamos a modelagem de equações estruturais para investigar os efeitos da cobertura florestal sobre a qualidade da água dos hábitats e na composição larval de anofelinos em diferentes estações. Para os mosquitos adultos, realizamos a seleção de modelos lineares e não lineares para avaliar a resposta da riqueza e abundância de mosquitos à perda de floresta. Em resumo, encontramos fortes evidências que as larvas de anofelinos são afetadas pela qualidade da água do habitat e que as variáveis limnológicas exercem papel importante para a presença de certas espécies. Além disso, a floresta possui papel importante nesses ambientes, afetando direta e indiretamente a assembléia de anofelinos nos habitats em ambas as estações. Por outro lado, no ambiente terrestre os mosquitos sofrem influência da cobertura florestal, com o aumento da abundância total. Entretanto, as espécies vetoras são beneficiadas com a diminuição da cobertura florestal e o inverso é observado para as espécies não vetoras.

# Introdução Geral

Os processos de urbanização, perda da vegetação nativa e a modificação do habitat vem alterando em grandes porporções as florestas tropicais em todo o mundo (Malhi et al., 2008; Achard et al., 2020). Os efeitos dessas mudanças no uso da terra levam a perdas de biodiversidade e serviços ecossistêmicos, afetando a dinâmica populacional de mosquitos vetores com consequências importantes para a saúde pública (Steiger et al., 2016; Tadei et al., 2017; Barlow et al., 2018; Laurance et al., 2018). Conversões de ambientes naturais em outros usos são bastante preocupantes nos trópicos, pois as mudanças de paisagem (como desmatamento) são seguidas por aumento de animais de produção, particularmente gado de corte (Rivero et al., 2009, Venturieiri et al., 2013, Domingues et al., 2014). Em algumas regiões como o Cerrado, este número deve aumentar drasticamente nos próximos anos, particularmente devido demanda internacional de carne e grãos (Ganem, 2015; Melz et al., 2014). Essas conversões ambientais propiciam a formação de habitats favoráveis à proliferação de mosquitos transmissores de infecções humanas (Norris, 2004).

Os mosquitos são os principais vetores de doenças humanas, transmitindo globalmente mais de 17% de todas as doenças infecciosas (Conass, 2019). A relação entre o desmatamento e a dinâmica de doenças transmitidas por mosquitos vetores é complexa e interligada. Por exemplo, o desmatamento e a urbanização aumentam o número e a distribuição de habitats disponíveis para o vetor da malária na Amazônia, expandindo a transmissão da doença (Tadei et al., 2017). Essas modificações ambientais favorecem o aumento do contato entre humanos e espécies de vetores (Santos & Almeida, 2018), entretanto, o efeito do número elevado de casos da doença em uma determinada região, podem induzir a redução do desmatamento por meio de mecanismos socioeconômicos (MacDonald & Mordecai, 2019).

A atividade humana na floresta contribui para o surgimento de novos tipos de criadouros, como represas, tanques de piscicultura e áreas desmatadas (Rodrigues et al., 2008; Vittor et al., 2009; Ferreira et al., 2015). A caracterização desses novos tipos de criadouros contribui para o conhecimento da dinâmica populacional de culicídeos, biologia e ecologia, principalmente a relação entre fatores ambientais e a presença do vetores na região (Tadei & Dutary-Thatcher, 2000; Piyaratne et al., 2005). Modelos preditivos baseados em variáveis ambientais têm sido utilizados como ferramentas importantes para fornecer informações sobre a dinâmica das espécies de mosquitos e seus impactos na Saúde Pública (Wilke et al., 2017). Além disso, estudos demonstram forte influência de variáveis ambientais como clima, pluviosidade, cobertura florestal e qualidade da água do habitat larval, na dinâmica de transmissão de doenças, dispersão, aumento populacional de mosquitos e presença de diferentes espécies vetores e não vetores (Lowe et al., 2011; Wolfarth et al., 2013; Arcos et al., 2018).

Nosso conhecimento é ainda limitado em identificar os níveis de transformação que geram mudanças críticas nos sistemas ecológicos (Wilson et al. 2016) ou seja, se as comunidades de mosquitos se alteram gradualmente (seguindo uma dada tendência) ou se as alterações são drásticas e repentinas. Tais sistemas ecológicos são vulneráveis à mudança irreversível, quando as propriedades-chave do sistema são empurradas sobre limiares. Talvez o mais importante dessas propriedades seja a quantidade total de vegetação nativa remanescente (Pardini et al. 2010). Vários estudos vêm focando na identificação de respostas não-lineares de elementos da biodiversidade nas quais o sistema tem um súbito limiar de alteração (Banks-Leite et al. 2014, Ochoa-Quintero et al. 2015, Rodrigues et al. 2016). Entretanto, nem todas as respostas da biodiversidade às mudanças ambientais exibem limiares singulares, que aparentemente ocorrem em certas porções do gradiente ambiental (geralmente quando a perda de habitat ultrapassa os

60%) e são modulados pela configuração do ambiente (Pardini et al. 2010). Além disso, o formato e dinâmica da matriz (áreas agrícolas), as variáveis ambientais, climáticas e a quantidade e tipo de animais domésticos podem desempenhar um papel importante na resposta de componentes da comunidade de mosquitos zoófilos e antropófilos. Neste contexto, esta tese está estruturada em três capítulos:

No Capítulo 1, estudamos os habitats aquáticos de mosquitos anofelinos com o uso de variáveis limnológicas, entomológicas e estruturais para identificar a relação dessas variáveis com a presença larval de *Anopheles*. O objetivo deste estudo foi caracterizar o habitat larval de anofelinos e relacionar os parâmetros ambientais com a presença larval dessas espécies, especialmente do principal vetor da malária na Amazônia. No Capítulo 2, usamos a modelagem de equações estruturais para investigar os efeitos da cobertura florestal sobre a qualidade da água dos hábitats e na composição larval de anofelinos em diferentes estações. Neste estudo tivemos como objetivo principal avaliar como a perda de floresta pode afetar direta e indiretamente (por meio de variáveis limnológicas) as assembléias de *Anopheles* em Manaus, Amazonas. No Capítulo 3, usamos a seleção de modelos lineares e não-lineares para avaliar a resposta da riqueza e abundância de mosquitos à perda da florestal nativa em uma área de Cerrado no Planalto da Bodoquena. O objetivo deste capítulo foi identificar o efeito da perda de vegetação nativa ao longo de um gradiente de cobertura florestal sobre a riqueza e abundância de culicídeos, e identificar potenciais limiares para cada espécie.

Esperamos que os resultados desta tese possam ser úteis para o conhecimento da biodiversidade de mosquitos na Amazônia e no Cerrado, além de elucidar os efeitos das modificações ambientais sobre a comunidade de culicídeos, em especial os vetores de doenças e suas implicações para a saúde pública no Brasil.

# Chapter 1

## Characterization of artificial larval habitats of *Anopheles darlingi* (Diptera: Culicidae) in the Brazilian Central Amazon

### Abstract

Mosquitoes belonging to the *Anopheles* genus are of great relevance in the epidemiology and transmission of malaria, with their larval phase developing in clean waters in the presence of organic matter. The human presence in the Amazon has increasingly influenced the emergence of new breeding sites and larval habitats, such as clay pits, fish ponds and dams, among others. The objective of the study was to characterize mosquito larval habitats using the biotic and abiotic parameters in the metropolitan area of Manaus. We collected in 23 artificial larval habitats in Manaus, classified in dams, fish ponds and clay pits. Water samples, *Anopheles* larvae, aquatic macrophytes and limnological parameters were collected from each artificial larval habitat. The Larvae Index per Man/Hour and canonical correspondence analysis were used for data analysis. Results indicate that larval habitats with characteristics similar to natural sites present higher larval density, displaying a high abundance of *An. triannulatus* and *An. darlingi*. Morethan 90% of the determined limnological parameters were in agreement with the environmental resolution stipulated by the CONAMA, while pH, dissolved oxygen and phosphorus levels were below the established limits at some of the larval habitats. Conductivity, total suspended solids and phosphorus were positively correlated to the presence of *An. albitarsis, An. peryassui* and *An. nuneztovari* in fish ponds, and *An. trianulatus* and *An. braziliensis* in dams. Thus, the evaluated limnological variables and habitat structure explain *Anopheles* species distribution in artificial larval habitats in the metropolitan Manaus region.

*Chapter published in Revista Brasileira de Entomologia - see Appendix

## Introduction

Malaria is one of the most epidemiologically important parasiticdiseases in the world, due to the high number of cases and deaths, mainly affecting the African continent (WHO, 2016; Stevenson & Norris, 2017). In Brazil, states located in the Amazon region exhibita higher incidence of this disease compared to other areas, totaling approximately 99% of the registered cases, with some reports from other regions (Brazil, 2017). Certain factors contribute to the maintenance of this vector, and, consequently, malaria, in the Amazon, such as the presence of a tropical forest, which shows favorable geographical and ecological characteristics for the interaction of the *Plasmodium* parasite and *Anopheles* mosquitoes, socio-cultural population conditions and migratory movements (Tadei et al., 1998; Martens & Hall, 2000).

In Brazil, the subgenus Nyssorhynchus is responsible for perpetuating malaria in the Amazon region, while Kerteszia is responsible for transmission in areas with Atlantic Rainforest coverage (Branquinho et al., 1997; Forattini, 2002; Rebêlo et al., 2007). The main malaria transmitter in the Amazon region is *An. (Nys.) darling*i Root, 1926 (Tadei et al., 2017) and other species, such as *An. (Nys.) albitarsis* s.l. Arribálzaga, 1878 (Consoli and Lourenço-de-Oliveira, 1994), *An. (Nys.) nuneztovari* Gabaldon, 1940, *An. (Nys.) triannulatus* Neiva and Pinto, 1922, *An. (Nys.) braziliensis* Chagas, 1907 (Tadei & Dutary-Thatcher, 2000), are considered occasionalor secondary vectors. Mosquitoes use natural and artificial water bodies, such as ponds, riverbanks, puddles and soaked fields, for oviposition and the development of the larval phase, preferably in clean waters, containing organic matter, aquatic vegetation and shading (Manguin et al., 1996a; Rejmankova et al., 1999). Human activity in the Amazon has led to the emergence of new types of breeding sites and larval habitats, such as dams, fish farming tanks, clay pits and deforestated areas (Rodrigues et al., 2008; Vittor et al., 2009; Ferreira et al., 2015).

Limnological and structural investigations of Culicidae larval habitats still require effort and monitoring, since each environment displays distinct and regional characteristics. For anophelines, environmental factors, such as light and temperature, directly affect the metabolism and development of immature larval stages, and also aid in the proliferation of algae and other organisms that are a staple part of the Culicidae diet (Forattini, 1962; Bergo et al., 1990).

The understanding of the relationship between the aquatic phases of the vectors and the environment in which they develop is extremely relevant for malaria control (Molineaux, 1997). The characterization of these new types of breeding sites contributes to knowledge on anopheline population dynamics, biology and ecology, mainly the relationship between environmental factors and the presence of mosquito larvae in these new larval habitats (Tadei & Dutary-Thatcher, 2000; Piyaratne et al., 2005; Ferreira et al., 2015).

This knowledge provides information that can influence or determine the location of oviposition and, the survival and, spatial and temporal distribution of these medically important species. Furthermore, this may also aid in integrated control strategies for these new habitats. Thus, the study sought to characterize the larval habitat of *Anopheles* spp. and to relate environmental parameters tothe larval presence of these species, especially the vector of malaria in the Amazon.

## Methods

### *Study area*

Anopheline samplings were carried out in the metropolitan area of the city of Manaus, at three types of artificial larval habitats (dams, fish ponds and clay pits) located on Highway AM-10 (02°53’44”S; 59°54’59”W), Cacau Pirêra AM-070 (03°10’18”S; 60°05’41”W) and Puraquequara/Brasileirinho (03°03’23”S;59°53’84”W), in the dry seasons of 2011 and 2012 (Figure 1).

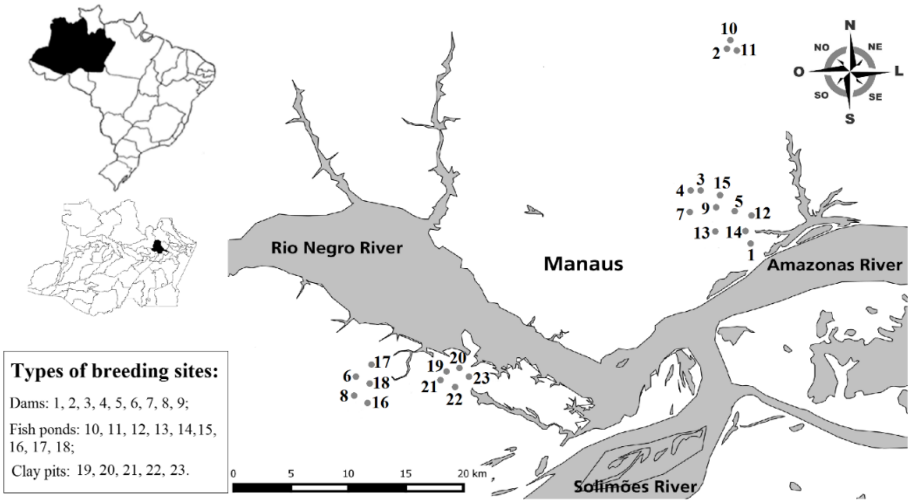


Figure 1. Distribution of artificial larval habitats in the metropolitan area of Manaus. The map was created using QGIS 3.16.5 (https://download.qgis.org)

### *Description of the artificial larval habitats*

The investigated fish ponds are permanent and semi-permanent larval habitats, partially shaded and sunny with the presence of surrounding riparian vegetation, marginal vegetation and macrophytes, influenced by natural and spring lakes. These larval habitats present an average air velocity of 1.3 m/s, 29.5°C temperature and 79.3% relative humidity. These types of habitats are common in the rural and periurban areas in Manaus (Figure 2A).

The evaluated clay pits ("poças de olaria") are located near urban areas and the AM-070 highway, created by removing clay for brick manufacturing. These areas present scarce surrounding riparian forest, little marginal vegetation and large amounts of organic material in the water, giving it a cloudy aspect. Overtime, these puddles are filled with rainwater and become larval habitats for Culicidae. These habitats, classified as permanent and semi-permanent, are considered new niches to be colonized by *Anopheles*, with the following characteristics: sunny, with an average air velocity of 1.6 m/s, 32◦C temperature and 70.0% relative humidity (Figure 2B).

Dams display characteristics closer to natural environments. They are influenced by springs and formed by man-made barriers used to dam and increase water coverage of a certain area, whichis is then used for pisciculture and leisure activities and as a water supply. These areas present average air velocity of 1.5 m/s, 29.4◦C temperature and 80.1% relative humidity. Dams present a vast vegetation cover, with the presence of a ciliary forest, macrophytes and allochthonous material originating from the forest, and display favorable environmental characteristics for the establishment and development of several *Anopheles* and Culicidae species (Figure 2C).

Figure 2. Artificial larval habitats evaluated herein and their local structural characteristics: (A) fish ponds, (B) clay pits and (C) dams.

### *Anopheles larvae sampling*

The larvae were collected with the aid of standard ladle with 350 mL volumetric capacity, an 11 cm opening and 1 m handle, in order to reach the areas around the larval habitats for 30 min at each site. The sampled individuals were separated in containersby habitat type and collection date, and fed three times a week with macerated fish feed (Tetra Marine Saltware, Tetra®mixed 1:1with Gold Fish Color, Alcon®), with the aim of obtaining adults for identification (Scarpassa & Tadei, 1990; Oliveira et al., 2012).

Due to the great difficulty in rearing *Anopheles* larvae in the laboratory, a simple and effective methodology was developed fordevelopment to the adult phase without significant losses: larvae were maintained in simulated environments, in trays with water from their respective breeding grounds (containing algae and organic material the larvae feed on) and floating macrophytes belonging to the *Salvinia* genus. This methodology was effective in the rearing and maintenance of the larvae in the laboratory.

The sampled specimens were sent to the Malaria and Dengue Laboratory – LMD/COSAS/INPA, where they were kept under controlled temperature conditions, at 26 ± 2◦C, upper relative humidity of 70–80% and a 12:12 h photoperiod. The larvae were maintained until adult emergence for identification using dichotomous keys (Gorham et al., 1967; Faran, 1980; Faran & Linthicum,1981; Consoli & Lourenço-de-Oliveira, 1994).

### *Larval habitat water sampling*

Three water samples were collected from each larval habitatin the dry seasons of 2011 and 2012, stored in sterile bottles and taken to the Environmental Chemistry Laboratory at CODAM/INPA, to determine limnological parameters. Portable equipment (OrionpH 290A+, YSI Dissolved oxygen and VWR “EC METER” 2052) was used for the pH, dissolved oxygen, temperature and electrical conductivity measurements.

Nitrate and phosphorus determinations were performed using the ion chromatography technique and total suspended solids were evaluated by the gravimetric method. These limnological parameters, along side their respective values, are recommended by the Brazilian National Council of the Environment – CONAMA (Brazil, 2005), that establishes values for the maintenance of aquatic life in natural waterbodies, through environmental resolution no. 357/2005, based on the Standard Methods for Water and Wast water Examination (APHA, 1985).

### *Aquatic macrophyte sampling*

To determine the richness of the macrophyte species present in each larval habitat, specimens were collected from the emergent, submerged and floating vegetation. The material was assigned to the LMD/COSAS/INPA and identified through specialized literature (Pott and Pott, 2000; Lorenzi, 2000).

### *Structural, environmental larval habitat parameters and malária cases*

Information on the structure of the larval habitats was recorded on a field work sheet created to identify local characteristics, such as temperature, relative air humidity and wind speed, obtained with a Kestrel 3000 digital thermo-hygro-anemometer. The rainfall index (mm) was obtained directly from the automatic meteorological station installed in Manaus (A101) and requested through the National Meteorological Institute (INMET/Brasília). Positive cases of malaria were obtained from the epidemiological surveillance system and online information (VigWeb, 2017).

*Data analyses*

The Larvae Index per Man/Hour (LIMH), the relation between the number of captured larvae, the number of collectors, the number of hours and the number of collection sites, was calculated in order to estimate larval density (Tadei et al., 2007).

A canonical correspondence analysis (CCA) was applied to verify the effect of the evaluated environmental variables on the sampled anopheline species. The main objective of the CCA analysis is to identify the influence of environmental variables on patterns of species composition and abundance in a certain community. Analyses were performed on the R 3.0.2 statistical language with vegan package. The level of significance was set at 5% and the number of permutations used in the CCA was 999.

## Results

*Anopheles larvae*

All three types of larval habitats (fish ponds, clay pits and dams) were positive for the presence of anophelines, especially the vector of malaria in the Amazon. During the study, rainfall in the study área was high, averaging 203.8 mm, with a rainfall index at the larval habitats ranging from 51.8 to 253.4 mm per month.

A total of 2.863 specimens were collected, comprising tens pecies. The most abundant species were *An. triannulatus* 1.286(44.9%), *An. darlingi* 566 (19.7%) and *An. nuneztovari* 464 (16.2%). The dam larval habitats showed higher species abundance and richness. The larvae rate per man-hour was higher at dam's larval habitats one, two and eight, with 7.0, 8.2 and 7.1 larvae collected per minute respectively (Table 1).

Table 1. Number of individuals collected per species and larvae index per man-hour (LIMH*) in artificial larval habitats in the metropolitan area of Manaus.

| *Anopheles* species | Artificial larval habitats** | | | | | | | | | | | | | | | | | | | | | | | Total | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | | |
| *An. triannulatus* | 156 | 95 | 82 | 139 | 72 | 77 | 123 | 129 | 96 | 43 | 55 | 10 | 42 | 27 | 34 | 19 | 27 | 30 | 5 | 3 | 8 | 4 | 10 | 1286 | 44.9 |
| *An. darlingi* | 36 | 47 | 11 | 17 | 9 | 14 | 13 | 10 | 13 | 39 | 55 | 39 | 37 | 29 | 36 | 71 | 22 | 45 | 3 | 3 | 6 | 5 | 6 | 566 | 19.7 |
| *An. nuneztovari* | 10 | 23 | 5 | 9 | 11 | 18 | 22 | 15 | 17 | 25 | 22 | 33 | 11 | 29 | 19 | 17 | 30 | 33 | 22 | 17 | 31 | 29 | 16 | 464 | 16.2 |
| *An. albitarsis s.l.* | 1 | 3 | 9 | 2 | 16 | 5 | 6 | 11 | 5 | 0 | 2 | 2 | 13 | 8 | 15 | 12 | 16 | 9 | 9 | 1 | 2 | 1 | 3 | 151 | 5.2 |
| *An. nimbus* | 5 | 74 | 3 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 | 10 | 3 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 131 | 4.5 |
| *An. peryassui* | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 10 | 0 | 0 | 0 | 8 | 0 | 18 | 26 | 18 | 9 | 5 | 0 | 0 | 6 | 0 | 111 | 3.8 |
| *An. braziliensis* | 2 | 3 | 0 | 4 | 31 | 1 | 0 | 46 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 92 | 3.2 |
| *An. oswaldoi* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 3 | 24 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 2 | 35 | 1.2 |
| *An. evansae* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 8 | 0 | 0 | 0 | 2 | 1 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 0.7 |
| *An. deaneorum* | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0.1 |
| Total | 210 | 246 | 110 | 171 | 142 | 115 | 175 | 213 | 169 | 107 | 134 | 84 | 123 | 100 | 180 | 145 | 113 | 126 | 47 | 24 | 47 | 45 | 37 | 2.863 | 100 |
| LIMH* | 7.0 | 8.2 | 3.6 | 5.7 | 4.7 | 3.8 | 5.8 | 7.1 | 5.6 | 3.5 | 4.4 | 2.8 | 4.1 | 3.3 | 6.0 | 4.8 | 3.7 | 4.2 | 1.6 | 0.8 | 1.6 | 1.5 | 1.2 | | |

**Types of larval habitats: 1-9 dams, 10-18 fish ponds, 19-23 clay pits.

*Limnological parameters*

Over 90% of the evaluated parameters were within the values described in CONAMA Environmental Resolution n° 357/2005, that establishes values for the maintenance of aquatic life in natural water bodies (Table 2). Some fish ponds and clay pits presented pH, dissolved oxygen and total phosphorus values below the values recommended by this resolution.

No significant temperature variation between breeding sites was observed, with an average of 30.0 ºC. High electrical conductivity, total suspended solids, turbidity and nitrate values were recorded mainly in the fish ponds and clay pits, albeit within the limits established by CONAMA for lentic environments at all larval habitats.

Table 2. Limnological parameters analyzed in the artificial larval habitats of the Manaus metropolitan area.

| *Anopheles* larval habitats | Temp. °C | pH | DO mg/L | Cond. µS/cm | Turb. NTU | TSS mg/L | Nitrate mg/L | Phosphorus mg/L |
|---|---|---|---|---|---|---|---|---|
| 1 | 30.4 | 6.7 | 6.1 | 7.0 | 1.3 | 2.2 | 0.01 | 0.00 |
| 2 | 28.6 | 6.2 | 6.3 | 10.0 | 2.8 | 2.2 | 1.05 | 0.02 |
| 3 | 28.4 | 6.0 | 6.6 | 12.0 | 7.2 | 10.0 | 1.35 | 0.01 |
| 4 | 27.5 | 6.1 | 6.0 | 16.0 | 3.1 | 5.2 | 0.18 | 0.01 |
| 5 | 32.7 | 7.3 | 6.4 | 7.7 | 2.6 | 0.8 | 0.01 | 0.00 |
| 6 | 31.3 | 6.4 | 8.4 | 6.0 | 1.5 | 6.0 | 0.04 | 0.01 |
| 7 | 29.0 | 6.9 | 7.2 | 10.7 | 2.0 | 3.6 | 0.01 | 0.01 |
| 8 | 30.2 | 6.5 | 8.1 | 11.3 | 2.2 | 4.8 | 0.03 | 0.01 |
| 9 | 27.2 | 8.4 | 7.2 | 16.2 | 3.4 | 11.6 | 0.09 | 0.02 |
| 10 | 28.7 | 5.9* | 5.9* | 7.4 | 25.7 | 9.4 | 0.03 | 0.01 |
| 11 | 29.3 | 5.7* | 6.2 | 6.9 | 21.6 | 8.4 | 0.01 | 0.01 |
| 12 | 31.1 | 6.6 | 4.3* | 72.6 | 26.8 | 13.0 | 0.25 | 0.02 |
| 13 | 29.3 | 7.4 | 6.9 | 88.2 | 13.2 | 14.0 | 0.06 | 0.01 |
| 14 | 28.9 | 6.8 | 6.2 | 160.6 | 11.4 | 16.4 | 0.05 | 0.01 |
| 15 | 28.2 | 6.1 | 7.1 | 17.5 | 3.5 | 7.0 | 0.10 | 0.02 |
| 16 | 30.2 | 6.3 | 3.9* | 40.5 | 15.0 | 20.0 | 0.85 | 0.20* |
| 17 | 31.1 | 6.5 | 3.9* | 69.6 | 29.0 | 19.5 | 1.02 | 0.12* |
| 18 | 30.8 | 6.7 | 4.0* | 58.4 | 18.7 | 23.7 | 0.93 | 0.17* |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 19 | 30.6 | 6.3 | 3.6* | 46.1 | 13.5 | 18.2 | 0.09 | 0.01 |
| 20 | 31.3 | 6.3 | 2.3* | 97.6 | 37.3 | 37.4 | 0.14 | 0.10* |
| 21 | 31.2 | 7.5 | 4.0* | 52.5 | 24.4 | 69.6 | 0.30 | 0.09* |
| 22 | 31.4 | 6.1 | 3.9* | 91.3 | 32.1 | 58.3 | 0.06 | 0.12* |
| 23 | 30.9 | 7.2 | 2.6* | 77.9 | 29.9 | 25.1 | 0.19 | 0.01 |
| * CONAMA N° 357/2005 | - | 6.0- 9.0 | >6.0 | - | <40.0 | <500 | <10.0 | <0.020 |

*Values outside the standards recommended by the current environmental resolution.

### *Macrophyte richness*

Emerged, floating and submerged macrophytes were identified in all artificial larval habitats, totaling thirteen genera (Table 3). The genera *Utricularia* sp. and *Eleocharis* sp. were the most frequent in all larval habitats, with the dam displaying higher richness of species when compared to the other habitats. The most frequent genera were *Oriza* sp, *Xyris* sp, *Urosphata* sp, *Eichhornia* sp, *Marsilea* sp and *Nymphaea* sp.

Table 3. Presence and absence of aquatic macrophyte genera in artificial larval habitats in the metropolitan area of Manaus.

| Macrophytes | Dams | Fish ponds | Clay pits |
|---|---|---|---|
| *Utricularia* sp. | X | X | X |
| *Brachiaria* sp. | X | X | - |
| *Pistia* sp. | X | X | - |
| *Oriza* sp. | X | - | - |
| *Xyris* sp. | X | - | - |
| *Cyperus* sp. | X | X | - |
| *Urosphata* sp. | X | - | - |
| *Salvinia* sp. | X | X | - |
| *Cabomba* sp. | X | X | - |
| *Eichhornia* sp. | X | - | - |
| *Marsilea* sp. | X | - | - |
| *Eleocharis* sp. | X | X | X |
| *Nymphaea* sp. | - | X | - |

### *Relationship between environmental parameters and Anopheles*

The canonical correlation analysis (CCA) indicated significant positive correlations between *An. albitarsis*, *An. peryassui* and *An. nuneztovari* with conductivity (r = 0.4623, p = 0.021), total suspended solids (r = 0.7656, p = 0.001) and phosphorus (r = 0.7033, p = 0.003) in fish tanks (r = 0.2609, p = 0.01). Species *An. triannulatus* and *An. braziliensis* presented positive correlations with the dam larval habitat (r = 0.2609, p = 0.01), but not with any limnological parameter. The other parameters and anopheline species were not positively correlated (p> 0.05) (Figure 3).

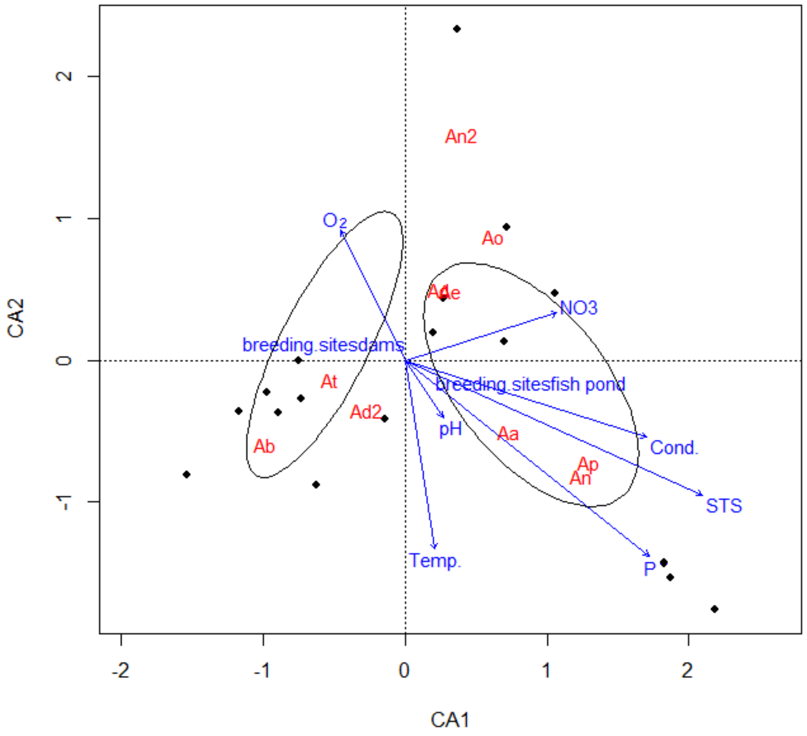


Figure 3: Ordering diagram of the canonical correlation analysis (CCA) between environmental factors "limnological parameters" and larval habitat type with *Anopheles* species: At (*Anopheles triannulatus*); Aa (*Anopheles albitarsis* s.l.); Ad (*Anopheles darlingi*); An (*Anopheles nuneztovari*); Ao (*Anopheles oswaldoi*); Ap (*Anopheles peryassui*); Ab (*Anopheles braziliensis*); An2 (*Anopheles nimbus*); Ad2 (*Anopheles deaneorum*); Ae (*Anopheles evansae*); DO (dissolved oxygen); NO3 (nitrate); pH (hydrogenionic potential); Temp (temperature); Cond (electrical conductivity); P (phosphorus); TSS (total suspended solids).

# Discussion

*Artificial larval habitats and entomological parameters*

The characterization of artificial larval habitats performed here in is the first study carried out in Manaus, aiming to relate limnological parameters to the *Anopheles* larval stage, identifying environmental factors associated to the presence of mosquitoes in these new niches. This characterization aids in identifying these relationships and provides data for tracing vector control models (Barbosa et al., 2008). Studies in Manaus point out the importance of artificial larval habitats for mosquito and malaria occurrences throughout the year, even during the dry season (Rodrigues et al., 2008; Tadei et al., 2017).

Positive cases peaks of autochthonous malaria in Manaus occurred at the dry season peak on the second half of two consecutive years (Figure 4). By October 2017, there were 3030 cases and one death, which indicates a large increase in relation to previous years (VigWeb, 2017).

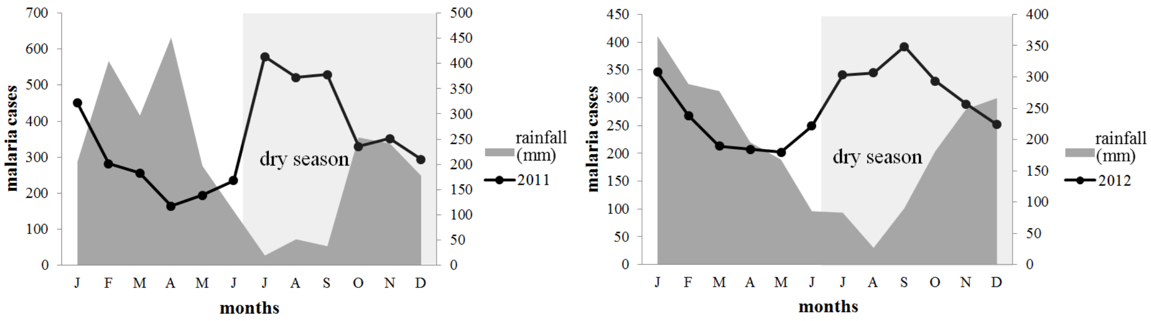


Figure 4: Monthly variation of malaria cases in relation to rainfall in 2011 and 2012 in the dry and rainy season in Manaus.

These artificial larval habitats present a relevant role in these cases of malaria regardless of the rivers level and amount of rainfall in the region, since they are active throughout the year, influencing the abundance of the vector mosquito *An. darlingi* in the dry season. However, in natural environments rainfall availability is a limiting factor in the abundance of mosquitoes (Fillinger et al., 2004), and also higher occurrences of *An. darlingi* are found in the intermediate (winter-summer) period in the Amazon (Tadei et al., 1993). And the fast increase in the abundance of these mosquitoes may hinder the development of effective vector.

Environmental, physical, chemical or ecological parameters become important in configuring an entire system present in a natural or artificial breeding site. Within this line of research, several authors have described the use of these parameters in verifying relations with anophelines (Manguin et al., 1996b; Tadei et al., 1998; Rubio-Palis et al., 2005; Xiao-Bo et al., 2012).

Some studies already cover these types of sites in the Amazon (Rodrigues et al., 2008; Vittor et al., 2009; Ferreira et al., 2015; Reis et al., 2015), indicating the presence of the malaria vector in the region. Environments like lagoons are more favorable habitats for the development of several *Anopheles* species (Rejmankova et al., 1999; Rubio-Palis et al., 2005), mainly due to the presence of macrophytes, creating a microhabitat that serves as a refuge against predators, favoring oviposition (Orr & Resh, 1989; Orr and Resh, 1992). This was observed in the investigated dams in the present study, since they share similar characteristics to natural environments, including the presence of macrophytes, water quality and shading.

The richness and abundance of anophelines in the evaluated larval habitats were higher in both the investigated dams and fish ponds. It is noteworthy that mosquitoes sought different oviposition and larval development sites, according to species requirements (Table 1, Figure 5).

These larval habitats may display certain specific features, such as clear water and a clean, shaded environment, with the presence of aquatic vegetation, algae and nutrients and be located near human dwellings. For example, *An. darlingi* develops in habitats with clean water, preferably dark and close to human dwellings, because this species presents anthropophilic behavior (Tadei et al., 1993). This behavior was also observed in the present study, with a higher abundance of anophelines in fish ponds and dams near human dwellings. It is noteworthy that these larval habitats are located in the periurban zone of Manaus, thus contributing to the continuance and increase of malaria cases in the area. This species, even when present in low densities, has the capacity to maintain the endemicity of malaria (Osório-Quintero et al., 1996; Silva-Vasconcelos et al., 2002).

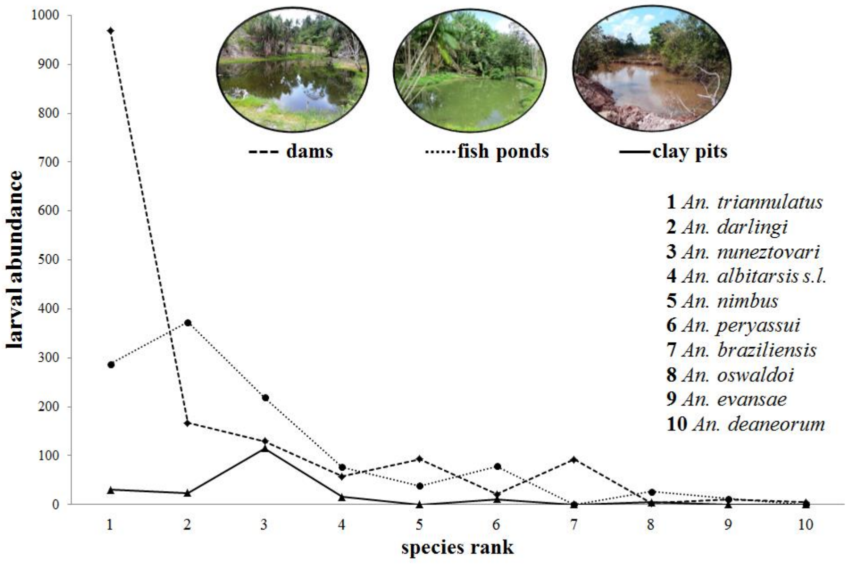


Figure 5: Ranking of the distribution of *Anopheles* larvae abundance in the three types of artificial larval habitats evaluated herein.

Most *An. darlingi* and *An. triannulatus* specimens were collected in environments displaying natural features (Table 1, Figure 5), which has also been observed in larval habitats in the state of Amapá (Galardo et al., 2009).

Similar results regarding the distribution of the most frequent species observed herein and the larval density of anophelines have been observed in other endemic malaria areas (Moreno et al., 2000; Berti-Moser, 2008). Some studies have recorded the presence of immature *An. triannulatus* and *An. darlingi* in different larval habitats (Rubio-Palis et al., 2005; Moreno et al., 2007; Ferreira et al., 2015). This behavior, also observed herein, may serve as an indication for the presence of these species in similar breeding sites, indicating their coexistence.

In addition, some secondary vector anophelines that also coexist with *An. darlingi* can also transmit human malaria. In the Amazon there is a possibility that *An. triannulatus, An. nuneztovari* and *An. oswaldoi* are secondary malaria vectors (Tadei et al., 1983; Arruda et al., 1986; Oliveira-Ferreira et al., 1990; Branquinho et al., 1996), since they distribution occurred herein, with the first two present in higher abundance. These species, along side *An. albitarsis* s.l. are found in urban and rural areas in the Brazilian Amazon, in both immature and adult forms (Tadei et al., 1993; Tadei et al., 1988).

The dam larval habitats it the present study displayed the highest larval density (LIMH), followed by fish ponds. Larval density values followed anopheline abundance and larval habitat structure, such as the presence or absence of macrophytes and limnological parameters. The LIMH aids in locating greater receptivity areas for *Anopheles* development, in addition to standardizing the sampling effort (Tadei et al., 2007).

In a study carried out in large altered areas in Manaus, the LIMH in clay pits was of 2.3 larvae per sampling minute, on average, while a value of 16.6 in fish ponds was recorded, displaying the highest density among the investigated environments (Rodrigues et al., 2008). In the present study, dams displayed the highest larvae rate per man-hour, followed by fish ponds and clay pits. However, relative abundance values in natural breeding sites are significantly higher, and can be explained by the environmental variables related to larval habitat type (Gouagna et al., 2012).

### *Limnological parameters*

There is growing interest in the investigation of limnological parameters as important components directly related to the presence and absence of certain *Anopheles* species. These environmental variables may also determine anopheline larval abundance (Kengluecha et al., 2005), and optimum temperature, algae, ammonia, nitrate and sulfate concentrations may affect the presence, development and survival of *Anopheles* larvae (Pal, 1945; Robert et al., 1998; Gimnig et al., 2001; Oo et al., 2002; Mutero et al., 2004; Low et al., 2016).

The limnological parameters determined in the present study were constant for each type of larval habitat. However, pH and dissolved oxygen were lower that values established by the CONAMA, especially in the investigated fish ponds and clay pits. These results are due to human changes, local environmental characteristics and, mainly, seasonality. Two distinct periods occur in the Amazon region, one with heavy rainfall and the other with a shortage of rainfall. In addition, it is noteworthy that one of the main biological characteristics of *Anopheles* larvae is the fact that they breathe mainly atmospheric oxygen (Consoli & Lourenço-de-Oliveira, 1994; Forattini, 2002), and, thus, do not depend on dissolved oxygen in the water, which is important for several other aquatic organisms.

Potential of hydrogen (pH) values in breeding sites under the influence of the Rio Negro, in Manaus, are altered, due to the acidic character of the water, which is not taken into account by the environmental resolution mentioned previously (Sioli, 1964; Arcos et al., 2016), indicating that regional characteristics must be respected and should be included in Brazilian classification standards.

The presence of *Anopheles* was recorded even in breeding sites displaying low plant cover and high temperatures, demonstrating the versatility of this species regarding abiotic parameters. The high temperatures observed in turbid water pools increase evaporation rates, reducing the permanence of these larval habitats in the environment, leading to the acceleration of the larval development (Paaijimans et al., 2008), especially in clay pits.

An average temperature of 27.2 ° C positively influences larval abundance (Devi et al., 2015), and successive temperature fluctuations may impair *Anopheles* larval development (Consoli & Lourenço-de-Oliveira, 1994). This explains the low larvae abundance recorded in the clay pits, as these are recent larval habitats, that do not display an abundance of ciliary forests, facilitating increased solar incidence and temperature increases (Table 2). The colonization of *An. darlingi* in new larval habitats in the Amazon is estimated at three years (Tadei et al., 1998), the required time for the natural succession process and the establishment of the water body to transpire. Another factor associated with *Anopheles* colonization is the emergence of vegetation at the edge of the breeding site, providing shade, shelter and microhabitats for *Anopheles* larvae, as well as other Culicidae.

Aquatic macrophytes belonging to the larval habitat structure serve as microhabitats and food items (Rejmankova et al., 1992; Marten et al., 1996) for *Anopheles* larvae and other aquatic invertebrates, and were present in 100% of the habitats evaluated herein. In addition, they use nutrients, such as phosphorus and nitrogen, for their growth, thus being considered indicators of nutrient increases in lakes, favoring anopheline reproduction (Forattini, 1962; Osorio-Quintero et al., 1996), as well as Diptera abundance (Peiró & Alves, 2006). The presence of macrophytes in the larval habitats studied has influenced the abundance and richness of *Anopheles*, being observed in the dams and fish ponds (Table 3).

### *Relationship between environmental parameters and Anopheles*

Biotic and abiotic environmental factors may influence the presence, development and larval abundance of *Anopheles* in different types of natural and artificial larval habitats, in different regions (Fillinger et al., 2004; Kengluecha et al., 2005; Reis et al., 2015). The correlation analysis pointed out phosphorus, total suspended solids and conductivity as the most important limnological variables in explaining the presence and distribution of *An. peryassui*, *An. nuneztovari* and *An. albitarsis* s.l. in fish ponds, indicating a positive correlation. The high values of these parameters in this type of larval habitat are related to the entry of allochthonous material and the addition of fish feed to the water, causing nutrient enrichment and leading to physic-chemical alterations. Some parameters, such as total suspended solids, cations and anions have been correlated with the occurrence of *Anopheles* in different habitats (Rejmankova et al., 1993).

An input of nutrients in these larval habitats increases the amount of total suspended solids and turbidity (Hussar et al., 2005; Rodrigues et al., 2008, Buzelli & Cunha-Santino, 2013). These nutrients are also a food resource for anopheline larvae, and act as nutritional support for phytoplankton growth, an anopheline food item that is also a water quality indicator (Forattini, 2002; Vidotti & Rollemberg, 2004).

In malaria vector breeding sites in Srilanka, only abiotic factors and structural characteristics of the breeding site indicate *Anopheles* preference for habitat types, not limnological parameters (Piyaratne et al., 2005). However, a positive correlation was observed between limnological parameters and larval habitat type in the artificial sites evaluated herein. With a clear preference of certain species for more natural habitats while others prefer nutriente rich sites with more artificial features. The positive and negative relation with dissolved oxygen values in larval habitats has been described as a determinant factor for the larval presence of both anophelines and other Culicidae

(Pinault & Hunter, 2012; Devi et al., 2015; Kudom, 2015). This limnological parameter was shown to be an important variable in the correlation analysis in dams, indicating a more natural larval habitat, with high oxygen concentrations when compared to the other habitats.

Environmental and limnological parameters, such as pH, debris, macrophytes, temperature, nutrients and total solids have been associated to anopheline larval presence in several countries (Rejmankova et al., 1993; Manguin et al. 1996b; Gimnig et al., 2001; Oo et al., 2002; Mutero et al., 2004; Kengluecha et al., 2005; Rubio-Palis et al., 2005; Xiao-Bo et al., 2012; Low et al., 2016). The relationship between organism-environment for *Anopheles* in the Amazon is being altered by several factors (Tadei et al., 1983; Tadei et al., 1993; Barata, 1995) and the creation of new ecological configurations through environmental changes is a result of anthropogenic effects of the ecosystem. These environmental modifications favor the establishment of the vector mosquito and the transmission of diseases (Guimarães et al., 2004), besides affecting circadian biology (Rund et al., 2016).

Dams and fish ponds are potential *Anopheles* artificial larval habitats, offering support for the establishment of anophelines, especially for the known malaria vector in the Amazon. Entomological and limnological characterizations increase the knowledge on the distribution of these medically important species, identifying environmental factors related to both the presence and absence of *Anopheles*. These environments contribute to increases in malaria cases in the region during the entire year, as well as to antagonistic relations, mainly in the urban and periurban areas, due to urban advances towards the forest. Control strategies directed at the larval stage in these larval habitats are extremely important, especially in Amazon rural and periurban areas, where a high concentration of these types of habitats is observed.

# Chapter 2

## Seasonality modulates the direct and indirect influences of forest cover on larval anopheline assemblages in western Amazônia

### Abstract

Serious concerns have arisen regarding urbanization processes in western Amazônia, which result in the creation of artificial habitats, promoting the colonization of malaria vectors. We used structural equation modelling to investigate direct and indirect effects of forest cover on larval habitats and anopheline assemblages in different seasons. We found 3,474 larvae in the dry season and 6,603 in the rainy season, totalling ten species and confirming the presence of malaria vectors across all sites. Forest cover had direct and indirect (through limnological variables) effects on the composition of larval anopheline assemblages in the rainy season. However, during the dry season, forest cover directly affected larval distribution and habitat variables (with no indirect affects). Additionally, artificial larval habitats promote ideal conditions for malaria vectors in Amazonia, mainly during the rainy season, with positive consequences for anopheline assemblages. Therefore, the application of integrated management can be carried out during both seasons. However, we suggest that the dry season is the optimal time because larval habitats are more limited, smaller in volume and more accessible for applying vector control techniques.

* Chapter published in Scientific Reports - see Appendix

## Introduction

Urbanization, loss of native vegetation and habitat modification have dramatically altered tropical forests globally (Achard et al., 2002; Malhi et al., 2008). These land use changes have led to losses of biodiversity and ecosystem services (Laurance et al., 2018; Barlow et al., 2018) and affected the population dynamics of vector mosquitoes with important consequences for public health (Steiger et al., 2016; Tadei et al., 2017). Mosquitoes are key vectors of human diseases, globally transmitting more than 17% of all infectious diseases. Dengue and malaria cause 440,000 deaths annually, and the large numbers of people infected often overloads healthcare systems (WHO, 2020). In Brazil, >99% of malaria cases occur in Amazônia, including 63,361 cases of malaria in the state of Amazonas in 2019 (Fiocruz, 2019; FVS, 2019).

Serious concerns have been raised about urbanization in western Amazônia (Richards & VanWey, 2015), one of the world's richest biodiversity regions, which houses more than 7,400,000 humans (IBGE, 2020). Urbanization and human expansion in the region have been increasing both in urban centers and in peri-urban areas (Chase, 1998; Sathler et al., 2009). The relationship between deforestation and malaria dynamics is complex and interconnected. For example, deforestation and urbanization increase the number and distribution of habitats available for the malaria vector *Anopheles darlingi* Root, 1926, thereby expanding malaria transmission (Tadei et al., 2017). These anthropogenic impacts also increase malaria cases resulting from increased contacts between humans and vector species (Santos & Almeida, 2018). In contrast, high numbers of malaria cases reduce deforestation through socio-economic mechanisms (MacDonald & Mordecai, 2019).

Disentangling the role of several mechanisms by which deforestation affects mosquito diversity, distribution and abundance in tropical forests is challenging, because forest losses likely have direct and indirect effects on larvae and adults. For example, forest loss can reduce anophelines in the subgenus *Kerteszia*, which depend on tree holes and bromeliads for larval habitats (Pina-Costa et al., 2014). The loss of native vegetation can also change microclimatic conditions, such as temperature and humidity, which in turn may affect mosquito population dynamics (Murdock et al., 2017). Land use changes may also influence mosquito species by reducing their host taxa, such as non-human primates, thereby reducing pathogen transmission (Pongsiri et al., 2009).

Deforestation and urbanization processes often create new artificial larval habitats, such as trash, dams, ponds, and clay pits, promoting colonization by mosquitoes, including malaria vectors in Amazônia (Ferreira et al., 2015; Arcos et al., 2018a). The amount of forest around artificial habitats also influences water quality variables, such as temperature, dissolved oxygen, sediments, and dissolved and suspended organic matter. Seasonality can also modulate water quality (Costa et al., 2019). For example, in the dry season, lentic habitats are reduced, leading to increased primary production and dissolved solids and decreased pH, which are positively correlated with the presence of *Anopheles* species in aquatic systems (Moreno et al., 2015; Barros et al., 2020). Therefore, distinguishing the direct (mediated only by forest loss) and indirect (forest loss effects on larval habitats) effects of forest loss on mosquito diversity and abundance is fundamental for understanding and predicting mosquito assemblages.

In this study we assessed how forest loss might directly and indirectly (through limnological variables) affect *Anopheles* assemblages in Manaus, Amazonas. We hypothesized that forest loss and limnological variables would affect mosquito assemblages in the rainy and dry seasons differently because of their differing effects on mosquito habitat conditions (Figure 1). We expected that the forest cover gradient and limnological variables would have stronger effects on larval assemblages during the dry season. Forests play a critical role in retaining moisture (including larval habitats) and filter some forest dependent species. Also, during the dry season, water levels are decreased and organic matter is concentrated, strengthening effects of limnological variables on mosquito assemblages.

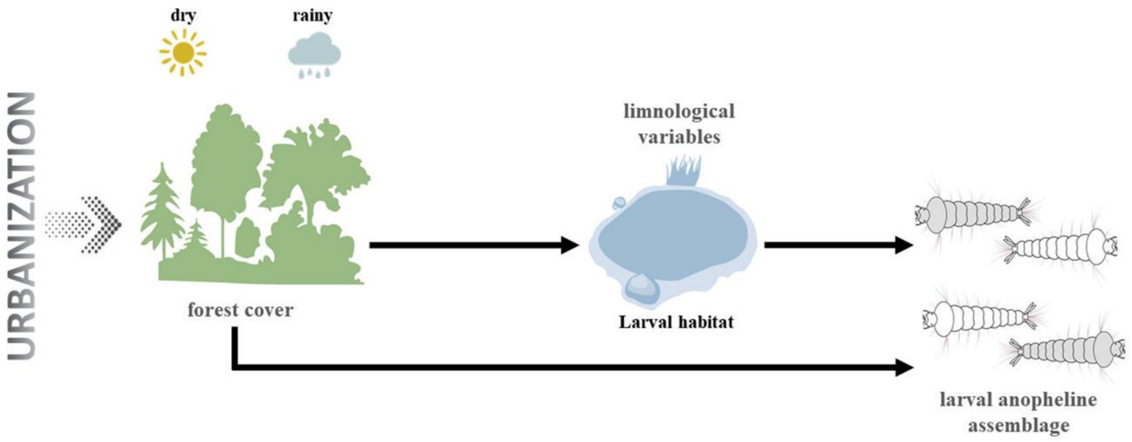


Figure 1. Conceptual structural equation model.

## Methods

### *Study area*

Manaus, the capital of Amazonas, has an area of 11,401,092 km² and an estimated population of 2,182,763 inhabitants (IBGE, 2020). It is located in central Amazônia, the world's largest tropical forest. The region has two seasons, a December-May rainy season with high volumes of rain (~30 cm per month) and a June-November dry season with little rain (~6 cm per month) (Farias et al., 2017) (Figure 2).

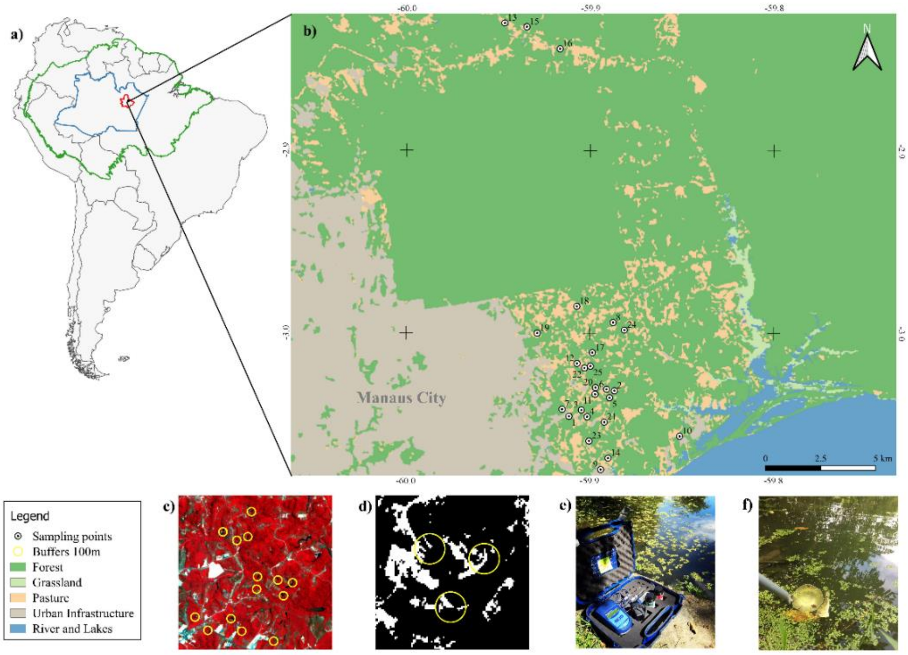


Figure 2. Locations of artificial larval *Anopheles* habitats in the Manaus periurban area. a) Amazon River basin with the delineation of Amazonas state and Manaus; b) sites, cover and land use; c) composition of bands 8-4-3 for vegetation classification; d) forest cover in 100 m buffers; e) collection of limnological variables; f) standard ladle for collecting larvae.

*Landscape analysis*

We built non-overlapping buffers of 100 m in radius around 25 larval habitats that were created by human modifications (e.g., clay pits and ponds) (Arcos et al., 2018) and estimated the proportion of forest within each buffer (Fig. 2; Supplementary Table S1). Biological knowledge of species dispersal are used to support the spatial extent (buffer size) used in ecological and entomological studies (Forattini, 2002; Miguet et al., 2016). Nonetheless, knowledge of dispersal movements of anopheline species is limited, so we used 100 m radii to avoid overlapping between neighbouring buffers. Moreover, 100 m represents an approximate mean of dispersion movement for some species (Greenberg et al., 2012), which is important for relating dispersion of adult

*Anopheles* from their larval habitats (Yakob & Yan, 2010). To classify land use, we used October 2016 images from the Sentinel-2 Level-1C sensor with a 10-m spatial resolution (ESA, 2015). After processing the images, bands 3, 4 and 8 (green, red and near infrared respectively) were merged to perform a semi-automatic classification in Quantun GIS version 3.4.13 - Madeira, using the Plugin SCP (Semi automatic classification plugin) version 6.4.0 of Greenbelt. The classification resulted in binary data (e.g., forest or non-forest) and classification accuracy was tested via the SCP Plugin in Quantum GIS using Google Earth images as references.

### *Anopheles larval sampling*

Larvae were collected in 2015 and 2016 one time each season for 30 min at each site by using a standard ladle with 350 mL volumetric capacity and a 1-m cable (Figure 2 f). The larvae were fixed in McGregor solution and sent to the Laboratório de Malária e Dengue of the Instituto Nacional de Pesquisas da Amazônia (INPA) for identification. Collections were authorized under SISBIO permit 21264/5 and larvae were identified by using taxonomic keys (Gorham et al., 1967; Faran, 1980; Faram & Linthicum, 1981; Consoli & Lourenço-de-Oliveira, 1994).

### *Abiotic variables*

Water samples were collected in sterile flasks and sent to the Laboratório de Química Ambiental (INPA), for filtering, drying and weighing total suspended solids (APHA, 1985). At each site, we used portable Orion pH 290A and YSI dissolved oxygen meters to measure pH, dissolved oxygen and water temperature in situ (Supplementary Fig. S1) (Figure 2 e). Daily precipitation amounts (mm) were obtained from the Manaus automatic meteorological station (BDMEP A101) of the National Institute of Meteorology (INMET, 2020).

*Data analyses*

To compare rainfall (mm) between seasons, we first run a Shapiro-Wilk normality to select an appropriate analysis. Based on the non-normality of the data, we used the Kruskal-Wallis test. We also used two ordination techniques to summarize both environmental variables and anopheline larval assemblage composition. To summarize, the variance of environmental variables in a reduced space, we used Principal component analysis (PCA). The scores of the first two PCA axes that captured most of variation were used to depict gradients in environmental variables (predictors). Principal coordinate analysis (PCoA) was used to summarize anopheline larval composition into a low dimensional space. Unlike PCA, which preserves Euclidean distance between objects, PCoA ordinates objects on the basis of any resemblance index, which is more aproproate to count data. We used Hellinger distance as a dissimilarity measure of assemblage composition, which produces good representation of objects in ordination techiniques (Legendre & Gallagher, 2001). The scores of the two PCoA axes were used to represent variation in anopheline larval composition (response variables).

To compare rainfall (mm) between seasons, we used the Kruskal-Wallis test. The choice of this non-parametric analysis was based on the Shapiro-Wilk normality test. We also used two ordination techniques to summarize both environmental variables and anopheline larval assemblage composition. Principal component analysis (PCA) was used to summarize, in a low-dimension space, the variance of environmental variables into orthogonal axes; the scores of the first two PCA axes were used to depict gradients in environmental variables (predictors). We used principal coordinate analysis (PCoA) to summarize anopheline larval assemblage composition into orthogonal axes. Unlike PCA, which preserves Euclidean distance between objects, PCoA ordinates objects on the basis of any dissimilarity, allowing more flexible handling of ecological data, such as counts. We used Hellinger distance as a dissimilarity measure of assemblage composition, which is ideal for linear models. The scores of two PCoA axes were used as response variables.

Percent forest cover, PCA and PCoA axes were used to create a causal model (Figure 1) that was tested using structural equation modeling (SEM) (Grace, 2006). SEM is a useful framework for revealing causal relationships between predictor and response variables, explicitly including theory *a priori* (Figure 1) (Grace et al., 2010). In this framework, we compared patterns in the data to those implied by the *a priori* model, seeking to minimize difference between the model predictions and observed data. All relationships were modelled using Gaussian linear relationships. We used the maximum likelihood chi-square formula and the associated p-value to test model adequacy. The model would be considered suitable for our data when $p > 0.05$. Individual path coefficients were assessed using z-scores and p-values. The analyzes were performed in R, using the Lavaan package (Rossel, 2012; R Core Team, 2016).

## Results

In the dry season, we collected 3,474 individuals and 6,603 individuals were collected in the rainy season. The most abundant species in both seasons were *A. triannualtus* (45.9 and 40.6%), *A. darlingi* (27.8 and 28.7%) and *A. nuneztovari* (9.4 and 13.5%) respectively. The malária vector, *A. darlingi,* was present in all larval habitats (Table 1).

Table 1. Seasonal abundance distribution of *Anopheles* larvae in 25 larval habitats in the Manaus periurban area.

| Species | Abundance (%) | |
|---|---|---|
| | Dry | Rainy |
| *A. (Nyssorhynchus) triannulatus* (Neiva & Pinto, 1922) | 1595 (46%) | 2686 (41%) |
| ***A. (Nyssorhynchus) darlingi* Root, 1926** | 971 (28%) | 1899 (29%) |
| *A. (Nyssorhynchus) nuneztovari* Gabaldon, 1940 | 330 (9%) | 894 (14%) |
| *A. (Nyssorhynchus) albitarsis* s.l. Lynch-Arribálzaga, 1878 | 151 (4%) | 350 (5%) |
| *A. (Stethomyia) nimbus* (Theobald, 1902) | 209 (6%) | 315 (5%) |
| *A. (Anopheles) peryassui* Dyar & Knab, 1908 | 121 (3%) | 212 (3%) |
| *A. (Nyssorhynchus) braziliensis* (Chagas, 1907) | 45 (1%) | 119 (2%) |
| *A. (Nyssorhynchus) oswaldoi* (Peryassú, 1922) | 38 (1%) | 75 (1%) |
| *A. (Nyssorhynchus) evansae* (Brèthes, 1926) | 14 (0.4%) | 46 (0.6%) |
| *A. (Nyssorhynchus) deaneorum* Rosa-Freitas, 1989 | 0 (0%) | 7 (0.1%) |
| Total | 3474 | 6603 |

Daily rainfall in the dry season ranged from 10.7 to 31.3 mm (mean= 26.6 ± 8.5 mm standard deviation) and in the rainy season, rainfall ranged from 235.3 to 303.9 mm (mean= 275.7 ± 29.7 standard deviation), indicating a significant seasonal difference in rainfall (Kruskal-Wallis, $x^2$= 37.857, df = 1, p<0.0001) (Supplementary Fig. S2).

The first two PCA axes explained 82% of the variation in water quality variables in the dry season and 80% in the rainy season. In both seasons, PCA1 captured a gradient of water quality, from sites with high dissolved oxygen concentration (negatively associated with PCA1) to those with high total suspended solids and pH (positively associated with PCA1). The most important PCA2 variable was water temperature (negatively associated in the dry season, positively associated in the rainy season) (Figure 3).

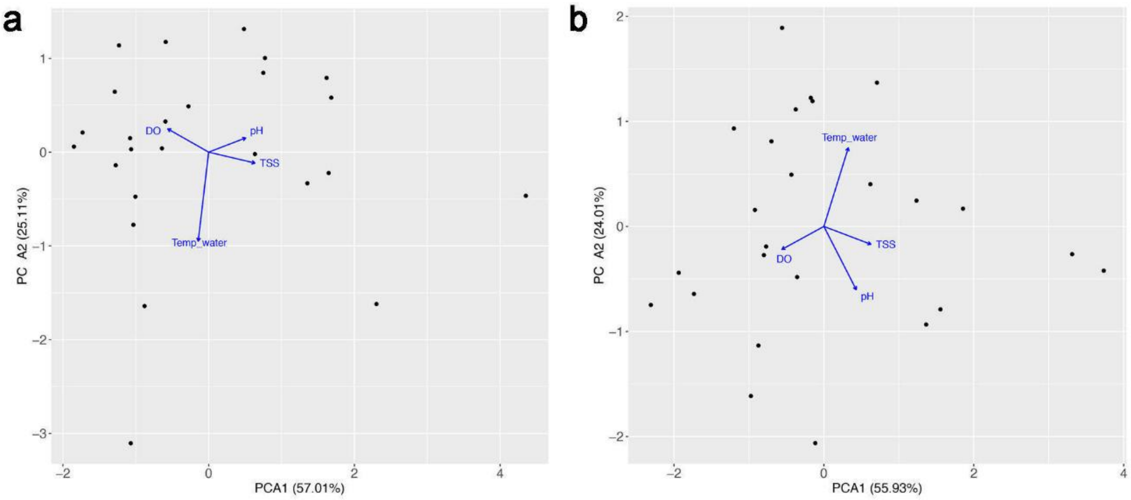


Figure 3. PCA of the limnological variables sampled in 25 sites in Manaus during the dry (a) and rainy (b) seasons. DO: dissolved oxygen; TEMP_WATER: water temperature; TSS: total suspended solids; Black points are sites.

The first two PCoA axes explained 52% of the variation in anopheline assemblages in the dry season and 60% in the rainy season. The most important species on the first axis in the dry season were *A. nuneztovari* (negatively associated) and *A. nimbus* (positively associated). In the rainy season, *A. nuneztovari* was also negatively associated with PCoA1, but *A. peryassui*, *A. nimbus*, *A. braziliensis* and *A. triannulatus* were positively associated with this axis. The most important species associated with PCoA2 in the dry season were *A. oswaldoi*, *A. evansae*, and *A. nimbus* (all negatively associated), but in the rainy season the most important species was *A. nimbus* (positively associated) (Figure 4). Thus, there was a clear seasonal difference in anopheline larval assemblages.

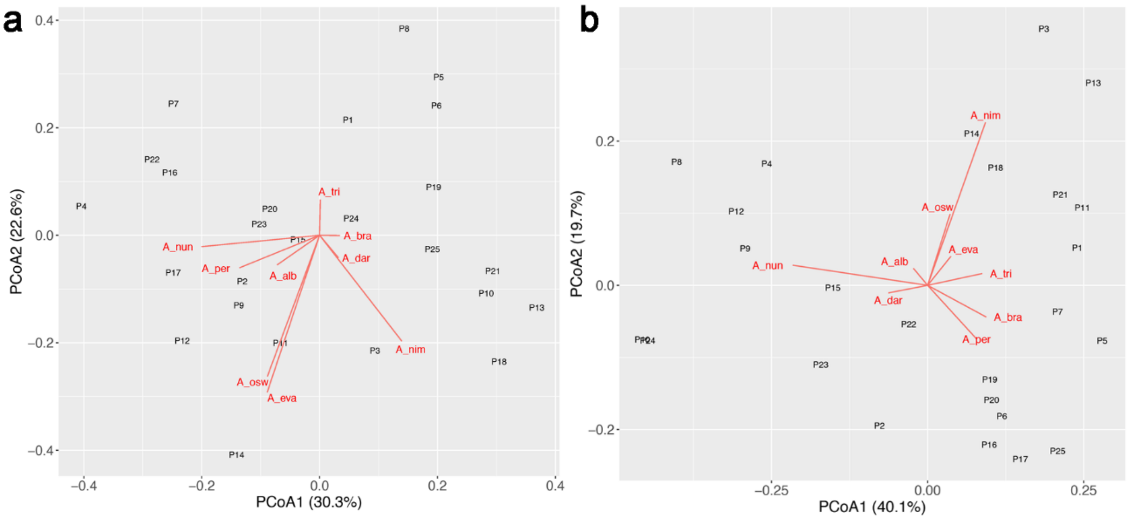


Figure 4. PCoA of anopheline larval assemblage composition in 25 sites in Manaus during the dry (a) and rainy (b) season. A_nun: *Anopheles nuneztovari*; A_per: *Anopheles peryassui*; A_alb: *Anopheles albitarsis*; A_osw: *Anopheles oswaldoi*; A_eva: *Anopheles evansae*; A_tri: *Anopheles triannulatus*; A_bra: *Anopheles braziliensis*; A_dar: *Anopheles darlingi*; A_nim: *Anopheles nimbus*; Numbers are sites.

In both seasons, the SEMs supported our hypotheses (dry season: $\chi = 0.071$; df = 1; p = 0.79; rainy season: $\chi = 0.000$; df = 1; p = 0.98) (Fig. 5). Forest cover negatively affected PC1 in the dry season (coefficient= -0.542, z= -3.227, p= 0.001, R2 = 0.294), meaning that increased forest cover was associated with decreased values along PC1 (sites with more dissolved oxygen). PC1 was negatively associated with PCoA1 (coefficient= -0.473, z= -2.179, p= 0.029), but did not affect PCoA2 (coefficient= 0.014, z= 0.062, p= 0.951). The PCoA1-PCA1 relationship means that greater levels of total suspended solids and pH were associated with increased numbers of *A. nuneztovati*, *A. peryassui*, *A. albitarsis*, whereas higher dissolved oxygen levels were associated with increased abundance of *A. nimbus*. The direct effect of forest cover on PCoA1 was insignificant (coefficient= -0.285, z= -1.307, p= 0.191), and the indirect effect of forest cover via PC1 was marginally significant (coefficient= 0.264, z= 1.834, p= 0.067). Forest cover did not affect PC2 (coefficient= 0.082, z= 0.414, p= 0.679), and PC2 did not affected PCoA1 (coefficient= 0.092, z= 0.500, p= 0.617) or PCoA2 (coefficient= 0.053, z= -0.269, p= 0.788). The direct effect and indirect effects of forest cover via PC2 on PCoA2 were also insignificant (direct: coefficient= -0.145, z= -0.617, p= 0.537; indirect: coefficient= 0.021, z= -0.093, p= 0.926). Variables used in this SEM explained 17% of PCoA1 and 3% of PCoA2 (Figure 5a).

For the rainy season, forest cover had a significant negative effect on PC1, i.e., increased forest cover was associated with decreased PC1 values (sites with more dissolved oxygen) (coefficient= -0.072, z= -3,972; p= 0.000; R2 = 0.387). Both direct and indirect effects (via PC1) of forest cover positively affected PCoA1 (direct: coefficient= 0.451, z= 3,853; p= 0.000; indirect: coefficient= 0.332, z= 2.973, p= 0.003). PC1 was negatively associated with PCoA1 (coefficient= -0.533, z= -4.561, p= 0.000), but did not affect PCoA2 (coefficient= 0.199, z= 0.837, p= 0.403). This relationship between PCoA1 and PC1 indicated that larger concentrations of dissolved oxygen increased the number of *A. nimbus*, *A. triannulatus*, *A. braziliensis*, *A. peryassui*, whereas greater values of total suspended solids, pH and water temperature increased the number of *A. nuneztovari* and *A. darlingi*. Forest cover did not affect PC2 (coefficient= -0.005, z= -0.025, p= 0.980), and PC2 did not affect PCoA1 or PCoA2 respectively (coefficient= 0.063, z= 0.688, p= 0.492; coefficient= -0.297, z= -1.597, p= 0.110). The direct effect of forest cover on PCoA2 was also insignificant (direct: coefficient= 0.276, z= 1.163, p= 0.245), as was its indirect effect (indirect: coefficient= -0.425, z= -1.498, p= 0.134). Variables used in this SEM explained 79% of PCoA1 and 13% of PCoA2 (Figure 5b). In summary, pH, dissolved oxygen and total suspended solids (but not temperature) affected anopheline assemblages in both seasons. Forest cover directly and indirectly affected anopheline assemblages in the rainy season, and had a marginal and indirect effect on anopheline assemblages in the dry season.

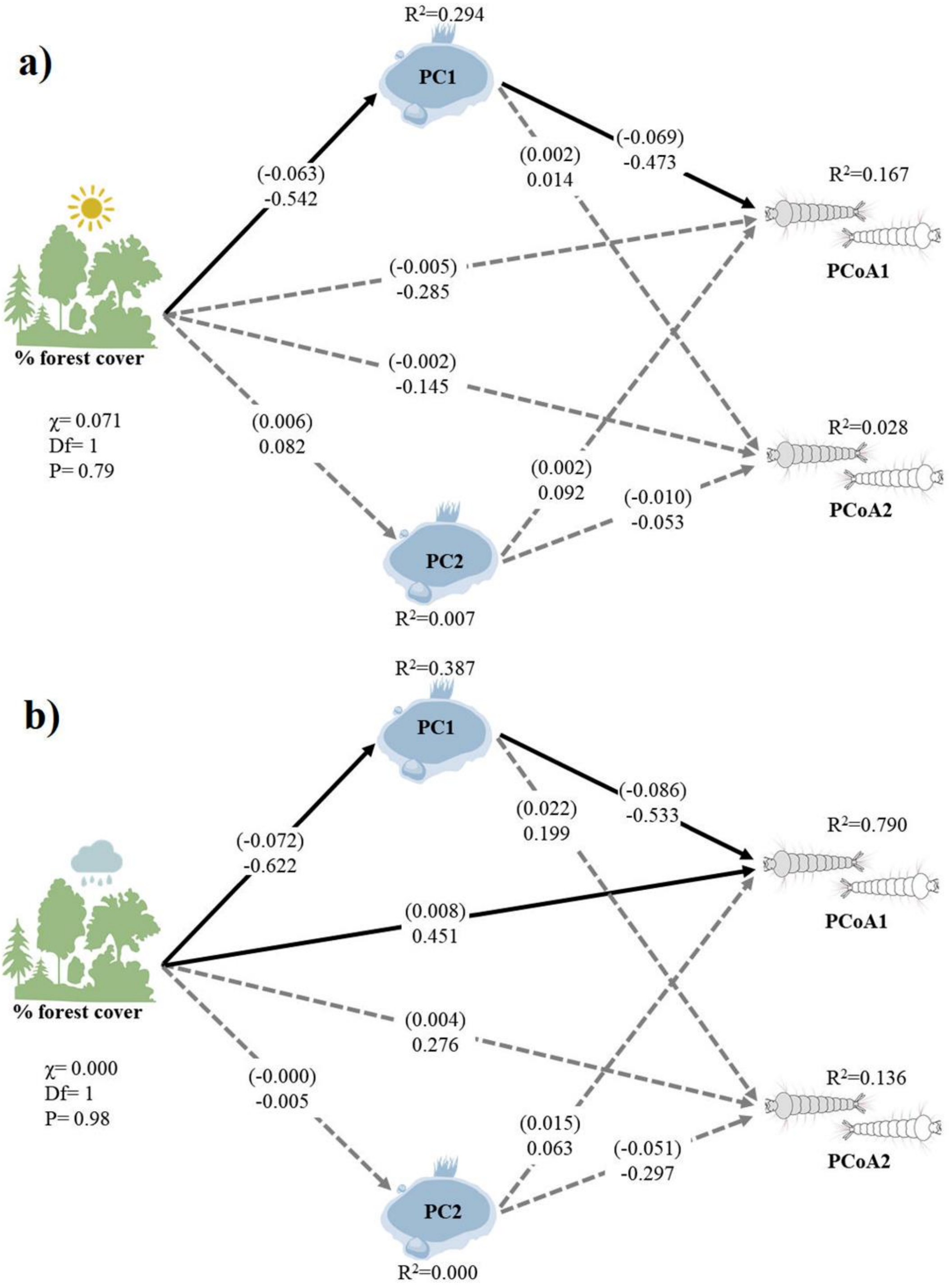


Figure 5. Schematic representation of dry (a) and rainy (b) season SEM results. The continuous arrows indicate significant paths and the dashed arrows indicate insignificant paths; PC1 and PC2 are the scores of the ordination of site water quality

variables and PCoA1 and PCoA2 represent larval anopheline assemblage composition captured by PCoA. R2 values are reported for each endogenous variable and non-standardized and standardized (in parentheses) coefficients are indicated on each path.

## Discussion

We untangled how the direct and indirect paths of forest cover and water quality variables interact and shape anopheline assemblages in two seasons. Although previous studies determined how environmental variables at different spatial extents affected anopheline distributions in Amazônia, most studies focused on a single effect of an environmental variable or focused on single habitat types (terrestrial or aquatic) (Moreno et al., 2015; Franklinos et al., 2019; Barros et al., 2020; Villarreal-Trivino et al., 2020). Our most important finding is that seasonality modulates the direct and indirect effects of forest cover on Amazônian anopheline larval distributions. In particular, we found that forest cover had stronger direct and indirect influence on larval anopheline assemblage composition in the rainy season than the dry season.

The different paths and strengths of forest cover influences on anopheline assemblages during the rainy and dry seasons can be associated with the responses of adults and larvae to forest characteristics. Forest cover influences water quality variables of ponds by shading, organic matter inputs and erosion processes (Birkinshaw et al., 2011). These effects have consequences for pond water quality (Arcos et al., 2018b) and favor the establishment of different culicid species (Emidi et al., 2017). We showed that during the rainy season, forest cover directly and indirectly influenced site water quality. Greater forest cover in the rainy season directly and indirectly affected *A. nimbus* and the secondary malaria vectors *A. triannulatus* and *A. braziliensis* positively. In the dry season, greater forest cover positively but marginally affected *A. peryassui, A. nuneztovari* and *A. albitarsi*s, but only indirectly through water quality. Some species like *A. triannulatus*, *A. nuneztovari* and *A. braziliensis* coexist with the malaria vector, *A. darlingi*, in breeding sites (Tadei & Dutary-Thatcher, 2000), and these species have been positively associated with pH, dissolved oxygen and total suspended solids in natural and artificial habitats Tadei et al., 1998; Arcos et al., 2018a), which are environmental conditions favored by greater forest cover. The marginal indirect effect of forest cover on anopheline assemblage in the dry season suggests that we need caution in the interpretation of this result and long-term temporal data is required to confirm if this effect is corroborated.

Forest conditions influence mosquito vectors and their hosts. For example, some mosquitoes are zoophiles that feed on the blood of birds, reptiles, and mammals (Vinod, 2011), which are often more abundant in conserved areas. Other species are anthropophilic and prefer to feed on human blood (Scott & Takken, 2012) and altered environments can force these species to migrate and, consequently, to change hosts (Vinod, 2011). In our study, *A. triannulatus* and *A. minbus* were more abundant in sites with more natural characteristics, whereas *A. darlingi* and *A. nuneztovar*i were more abundant in altered landscapes. In addition, urbanization and deforestation increase the proximities of humans and domestic animals to mosquito vectors and their hosts, thereby maintaining and increasing transmission cycles (Rucket & Ebel, 2018).

Forest conditions influence anopheline diversity by different paths, which may alter the strength of their seasonal effects. During the dry season, mosquito survival is also affected by altered microclimate (e.g., lower humidity) (Afrane et al., 2006) and lentic habitats contain less water, increased nutrient concentrations and decreased abundance and richness of mosquitoes (Mattah et al., 2017; Ferreira et al., 2020). We observed that rainfall plays an important role in the larval abundance of *Anopheles* in artificial larval habitats in Manaus. In addition, climatic factors such as rainfall and river levels are strongly associated with vector abundance and malaria cases in the region (Wolfarth-Couto et al., 2019; Galardo et al., 2009). During the rainy season, increased water volume in artificial habitats provides more areas for distribution and development of mosquito species (Coutinho et al., 2018) and we detected a significant increase in abundance of *A. triannulatus*, *A. darlingi* and *A. nuneztovari*. These observations may partially explain why we found a direct effect of forest cover on mosquitoes only during the rainy season.

Our results add more evidence that managing and conserving forest cover is important to control anophelines, thereby decreasing the contact of potential vectors (e.g., *A. darlingi*) with humans. In general, our results support the idea that mosquitoes are directly affected by the loss of native forest cover (Ferraguti et al., 2016) in the rainy season. Mosquitoes associated with serious human diseases (e.g., malaria, yellow fever, dengue, leishmaniasis) are more abundant in areas with low levels of native forest cover (WHO, 2014; Santos & Almeida, 2018). This is a critically important finding because recent studies have shown that forest cover plays an important role in the vector dynamics of mosquitoes and forest conservation keeps pathogens within the forest, avoiding spillover to human settlements (Ellwanger et al., 2020). On the other hand, deforestation provides favorable conditions for these vectors, thereby increasing malária cases and decreasing scores of the Human Development Index (Tchoumbou et al., 2020). In addition, there is a positive correlation between mosquito abundance in fragmented forests and the prevalence of *Plasmodium*, the protozoan that causes malaria (Rodrigues et al., 2008).

Artificial larval habitats promote conditions for malaria vectors in Amazônia (Rodrigues et al., 2008; Reis et al., 2018). Therefore, the best way to develop control techniques would be to understand larval ecology in these habitats, where they are more sensitive to infections by pathogens, parasites, predation, larvicides and growth regulators (Kamareddine, 2012). This information is necessary to minimize failures in programs to control or eradicate the vector and the disease. Under this perspective, our study adds a new piece in the puzzle of mosquito control in Amazônia. For example, during the rainy season when forest cover directly and indirectly influences larval habitats, control programs can strengthen the control of key limnological variables, habitat structure, and entomological aspects, intensifying the environmental filter, particularly in areas with little forest cover and greater human concentrations near those habitats. The limnological study of *Anopheles* larval habitats is still far from complete, as each case has peculiarities inherent to them. Despite attempts, anophelines demonstrate versatility in relation to abiotic parameters (Laird, 1988; Forattini, 2002; Arcos et al., 2018a; Arcos et al., 2018c).

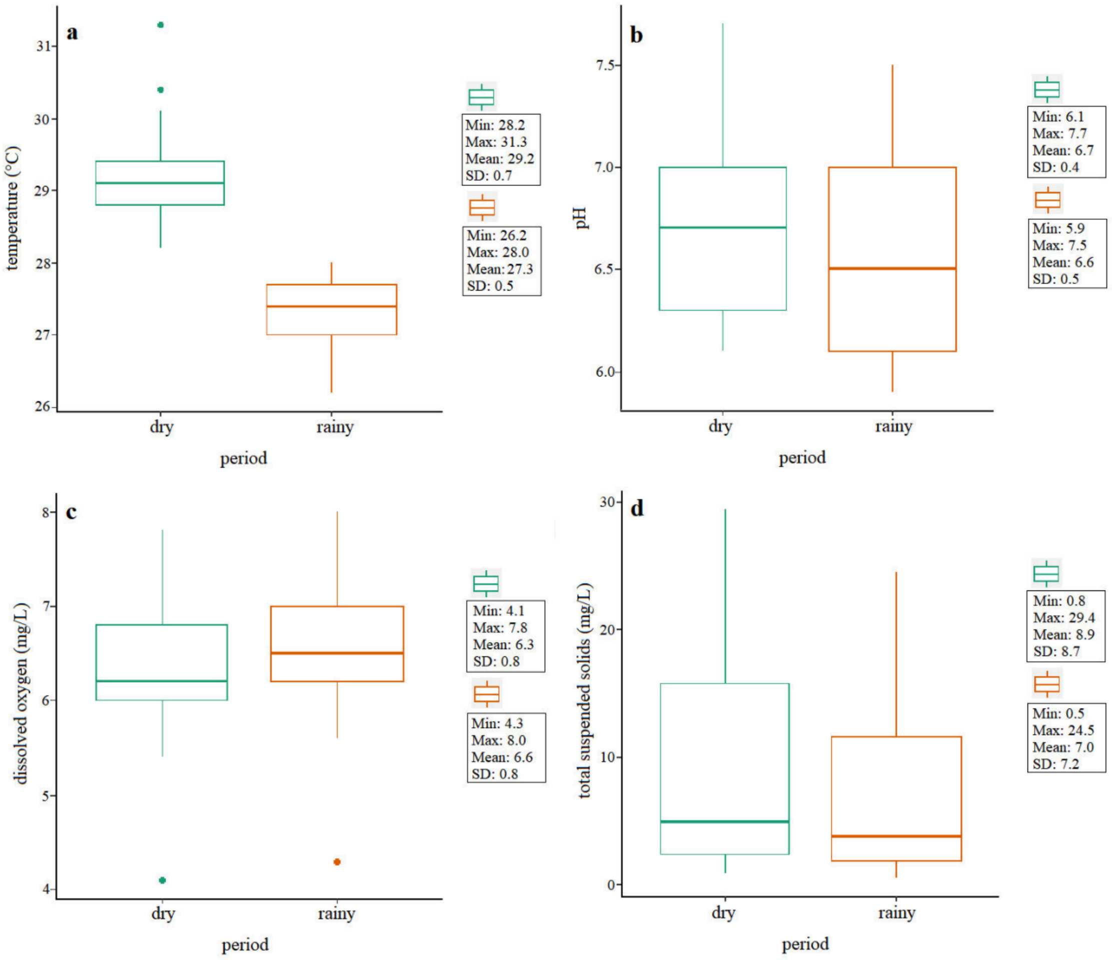
However, we can use approaches that modify the larval environments. For example, more efficient management of water levels in fish farming ponds could decrease larval numbers and anopheline reproduction, Similarly, greater rationing of fish feed would decrease the supply of food resources for mosquito larvae. It is also worth mentioning that some variables are related to the efficiency of others. Regarding biological control via entomopathogenic bacteria, environmental factors (solar radiation) and water quality (amounts of total suspended solids and organic matter), can interfere with the effectiveness of the formulated *Bacillus sphaericus* applied in habitats for vector control (Rodrigues et al., 2008; Souza et al., 2020). Furthermore, eutrophication decreased the assemblages of aquatic invertebrates predating mosquito larvae.

Another alternative is the use of physical control (removal of grasses and macrophytes from the edge of habitats), helping to reduce microhabitats that provide larval refuges. Also, increased light and water temperature at the edges favor natural predation and biological control processes from potential fish and macroinvertebrates. The conservation of natural enemies and the use of biotic agents in the population control of vector mosquitoes have been recommended in small and medium-sized natural and artificial breeding sites (Ferreira et al., 2015; Ferreira et al., 2020). A combination of techniques that shape the important environmental variables for the establishment of these species are essential for vector control.

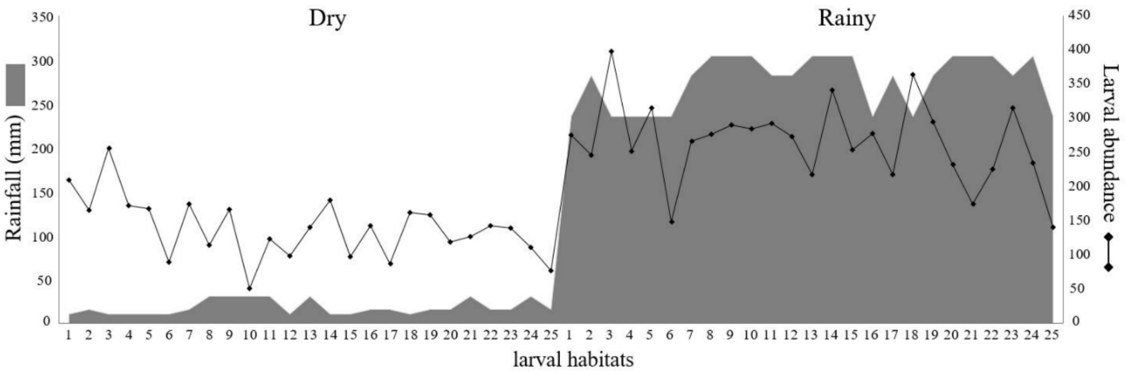
The analytical approach used here opens some windows of opportunity for improvements that are important to be recognized. First, our model did not incorporate important complexity of natural systems, such as ecological interactions among vectors and hosts, including human behavior. Agent-based models, including different host behavior, could provide important insights in this way. Second, our study is very limited in terms of temporal climatic variability. Additional information is needed to better understand the effects of long-term changes in land-use, water quality and climate and their interactions with mosquito assemblages in the region, particularly considering an ecological-evolutionary perspective. Third, it is important to highlight that the magnitude of effects of the estimated drivers were not the same in the rainy and dry seasons. Also, they may not remain constant in coming decades, especially considering potential regional process on mosquito assemblages, such as spillover effects, mass effects and host changes. Fourth, our study was carried out in an area of Amazonia that has experienced, a relatively old land use conversion from forest to urban areas (urban expansion rate of around 12% per year for the past 34 years) (Souza et al., 2020). Beginning in the 1970s, human population increased at a rate of around 23% per decade and 25% in Manaus (IBGE, 2020). Therefore, the region we studied is very relevant in terms of historical interactions among human populations, mosquitoes and land use changes. However, understanding the effect of these changes on mosquito assemblages in areas with different land-use change dynamics, provides us with important information (Norris, 2004), particularly those with very rapid urbanization processes, such as in the Arch of Deforestation (Sathler et al., 2018). Lastly, we need studies that consider the nexus among climate and land use changes, human and animal population health, economic conditions, and ecosystem services provided by these forest-urban transitional regions. Such information would facilitate including mosquito information in land use planning and climate mitigation programs based on forest management in and around cities.

# Supporting Information for Chapter 2

Supplementary Figure S1. Seasonal variations of water temperature (A), pH (B), dissolved oxygen (C) and total suspended solids (D) in artificial larval habitats.



Supplementary Figure S2. Seasonal rainfall amounts and larval abundances in artificial larval habitats.



Supplementary Table S1. Seasonal percentages of forest cover and *Anopheles* richness in larval habitats.

| Larval habitat | % Forest Cover | dry | rainy | dry | rainy | dry | rainy | dry | rainy | dry | rainy | dry | rainy | dry | rainy | dry | rainy | dry | rainy | dry | rainy |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *A. triannulatus* | *A. triannulatus* | *A. darlingi* | *A. darlingi* | *A. nuneztovari* | *A. nuneztovari* | *A. albitarsis* s.l. | *A. albitarsis* s.l. | *A. nimbus* | *A. nimbus* | *A. peryassui* | *A. peryassui* | *A. braziliensis* | *A. braziliensis* | *A. oswaldoi* | *A. oswaldoi* | *A. evansae* | *A. evansae* | *A. deaneorum* | *A. deaneorum* |
| 1 | 86,757 | 156 | 181 | 36 | 71 | 10 | 8 | 1 | 4 | 5 | 6 | 0 | 0 | 2 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 79,761 | 101 | 126 | 17 | 44 | 20 | 13 | 8 | 11 | 0 | 0 | 9 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 97,630 | 95 | 203 | 57 | 58 | 23 | 19 | 3 | 14 | 74 | 91 | 0 | 2 | 3 | 3 | 1 | 5 | 0 | 2 | 0 | 1 |
| 4 | 87,722 | 66 | 92 | 25 | 41 | 0 | 0 | 9 | 5 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 74,309 | 139 | 239 | 17 | 52 | 0 | 4 | 2 | 14 | 5 | 0 | 0 | 4 | 4 | 0 | 1 | 2 | 0 | 0 | 0 | 0 |
| 6 | 79,830 | 77 | 92 | 26 | 44 | 0 | 10 | 5 | 0 | 0 | 0 | 0 | 3 | 1 | 9 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 74,253 | 123 | 180 | 13 | 28 | 22 | 17 | 6 | 15 | 0 | 2 | 11 | 22 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| 8 | 52,748 | 96 | 120 | 13 | 36 | 0 | 0 | 5 | 9 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 63,132 | 55 | 77 | 55 | 81 | 22 | 18 | 25 | 26 | 4 | 8 | 6 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 48,793 | 10 | 35 | 39 | 44 | 0 | 23 | 2 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | 91,194 | 42 | 74 | 37 | 58 | 11 | 6 | 13 | 17 | 10 | 20 | 8 | 10 | 0 | 2 | 0 | 0 | 2 | 5 | 0 | 0 |
| 12 | 57,142 | 27 | 57 | 29 | 50 | 28 | 22 | 8 | 19 | 3 | 6 | 0 | 0 | 0 | 0 | 3 | 11 | 1 | 8 | 0 | 0 |
| 13 | 92,455 | 59 | 91 | 48 | 64 | 0 | 0 | 1 | 9 | 33 | 44 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 0 |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14 | 80,408 | 34 | 103 | 36 | 74 | 19 | 20 | 15 | 33 | 25 | 41 | 18 | 19 | 0 | 0 | 24 | 37 | 9 | 11 | 0 | 3 |
| 15 | 78,263 | 35 | 74 | 47 | 69 | 0 | 0 | 5 | 10 | 0 | 8 | 0 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | 82,195 | 19 | 91 | 71 | 104 | 17 | 13 | 2 | 16 | 0 | 0 | 26 | 30 | 8 | 15 | 0 | 0 | 0 | 9 | 0 | 0 |
| 17 | 85,165 | 27 | 88 | 62 | 90 | 30 | 22 | 1 | 10 | 0 | 0 | 18 | 35 | 5 | 11 | 3 | 7 | 0 | 2 | 0 | 0 |
| 18 | 72,079 | 50 | 99 | 71 | 81 | 0 | 29 | 1 | 14 | 42 | 50 | 9 | 16 | 11 | 19 | 0 | 0 | 0 | 3 | 0 | 0 |
| 19 | 82,637 | 89 | 148 | 52 | 94 | 0 | 15 | 3 | 19 | 1 | 0 | 2 | 4 | 4 | 9 | 6 | 2 | 2 | 2 | 0 | 1 |
| 20 | 80,188 | 56 | 126 | 43 | 68 | 9 | 16 | 5 | 5 | 0 | 0 | 6 | 11 | 0 | 2 | 0 | 4 | 0 | 0 | 0 | 0 |
| 21 | 96,931 | 47 | 60 | 65 | 82 | 0 | 0 | 10 | 23 | 5 | 8 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| 22 | 81,739 | 77 | 112 | 21 | 57 | 9 | 12 | 8 | 20 | 0 | 0 | 3 | 2 | 3 | 18 | 0 | 0 | 0 | 0 | 0 | 2 |
| 23 | 74,599 | 67 | 134 | 41 | 79 | 0 | 11 | 7 | 31 | 2 | 0 | 1 | 3 | 2 | 5 | 0 | 3 | 0 | 2 | 0 | 0 |
| 24 | 58,963 | 45 | 70 | 57 | 82 | 6 | 16 | 3 | 20 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | 82,954 | 24 | 54 | 44 | 62 | 0 | 0 | 3 | 3 | 0 | 0 | 4 | 10 | 2 | 8 | 0 | 2 | 0 | 0 | 0 | 0 |

# Chapter 3

## Responses of mosquito communities to a gradient of native forest cover loss in the Cerrado hotspot

### Abstract

Changes from natural landscapes have caused negative effects on biodiversity, population decline and a significant increase in the number of some mosquitoes, particularly of anthropophilic species. Our knowledge is still limited in identifying the levels of transformation that generate critical changes in ecological system, if the mosquito communities change gradually or if the changes are drastic and sudden. Perhaps the most important of these predictors is the total amount of native vegetation remaining. In this study we seek to identify the effect of native forest loss across a gradient of forest cover on the richness and abundance of vector and non-vector mosquitoes, and to identify potential thresholds for each species. The study was carried out in a Cerrado area - MS, and mosquitoes were collected in 2019. We use the selection of linear and non-linear models to assess the response of mosquito richness and abundance to forest loss. We registered a total of 6,910 mosquito specimens, and a positive effect of the amount of forest cover on the total abundance of mosquitoes in the landscapes. In addition, non-vector species are more susceptible to landscapes with low forest cover, negatively affecting the abundance of these species. On the other hand, the high abundance of vector species was associated with a low percentage of native forest cover. The thresholds values ranged from 12.5 to 81% of forest cover and presented different values for the 14 species. We emphasize that as deforestation increases in the region, there is a clear loss of species and an increase in the presence of potential disease vectors for animals and humans, being linked with potential implications for the emergence of arboviruses and in public health.

# Introduction

One of the most critical issues involving landscape changes in tropical environments is the dynamic of vectors of diseases such as mosquitoes. Due to the importance of disease-carrying mosquitoes in terms of public health, the ecological and environmental conditions that influence the abundance of these species are of public interest (Chaves & Koenraadt, 2010). Changes from natural landscapes to anthropogenic landscapes have caused negative effects on biodiversity, population decline in many species (Fahrig, 2003; Ewers & Didham, 2006), a significant increase in the number of some mosquitoes (Steiger et al., 2012), particularly of anthropophilic species (Medeiros-Sousa et al., 2017; Wilke et al., 2017).

Human interventions in the forests contribute to the displacement of vectors and their etiological agents, providing contact between wild animals and human populations and their domestic animals. These interactions have favored several zoonoses mainly in the area of settlements and peripheries (Carvalho et al., 2009). Although most scientists agree that habitat loss drastically decrease the populations of forest-dependent mosquito species, little is known about how these changes occur along landscape gradients, from the most preserved to the most altered environment. Conversions from natural environments to other uses are of great concern in the tropics. Landscape changes (e.g. deforestation) are followed by an increase in production animals, particularly cattle (Rivero et al., 2009, Venturieiri et al., 2013, Domingues et al., 2014). Brazil ranks first as an exporter of grains, meat and other products of animal and vegetable origin (Ganem, 2015), and this demand generates an enormous amount of land for production, resulting from deforestation. In some regions such as the Brazilian Cerrado, this number is expected to increase dramatically in the coming years, particularly due to the international demand for meat and grains (Melz et al., 2014; Ganem, 2015). And all these environmental conversions may provide the formation of habitats favorable to the proliferation of mosquitoes that transmit human infections (Norris, 2004).

Our knowledge is still limited in identifying the levels of transformation that generate critical changes in ecological systems (Wilson et al., 2016), that is, if the mosquito communities change gradually (following a given trend) or if the changes are drastic and sudden. Such ecological systems are vulnerable to irreversible change, when the key properties of the system are pushed over thresholds. Perhaps the most important of these predictors is the total amount of native vegetation remaining (Pardini et al., 2010).

Many studies have focused on the identification of non-linear responses of elements of biodiversity in which the system has a sudden threshold of change (Banks-Leite et al., 2014, Ochoa-Quintero et al., 2015, Rodrigues et al., 2016). However, not all biodiversity responses to environmental changes exhibit unique thresholds, which apparently occur in certain portions of the environmental gradient (usually when habitat loss exceeds 60%) and are modulated by the configuration of the environment (Pardini et al., 2010). In addition, the shape and dynamics of the matrix around the fragments (usually in the form of agricultural areas) and the quantity and type of domestic animals can play an important role in the response of components of the zoophilic and anthropophilic mosquito community. The thresholds of forest cover loss can cause a decrease in mosquito biodiversity and an increase in the abundance of vector species (Chaves et al., 2021).

With successive modifications of habitats, impacts on the dynamics of infectious diseases are expected, especially those associated with vectors and reservoirs in the forest, such as malaria, leishmaniasis and arboviruses (Githeko et al., 2000). Thus, the behavior of species associated with ecological factors allows for a better characterization of the interrelationships between vector species or potential vectors and human populations and their domestic animals that settle in a region (Tadei, 1986). Therefore, understanding how mosquitoes respond to landscape gradients is a priority to learn about the fluctuation of these populations and the potential for disease transmission, above all, can be key to planning more sustainable landscapes in the tropics, including reducing disease risks in humans and animals, maintaining biodiversity and also agricultural production.

In this study we seek to identify the effect of native vegetation loss across a gradient of forest cover on the richness and abundance of vector and non-vector mosquitoes, and to identify potential thresholds for each species. We hypothesize that the richness and abundance of mosquitoes will be shaped by native forest cover, with a positive effect on the abundance of vector species. In addition, considering that mosquito species may have different ecological requeriments and levels of dependence of forests (e.g. larval habitats, hosts) (Burkett-Cadena & Vittor, 2018), we expected that each species will respond differently to forest cover gradients, and that the sylvatic mosquitoes will be negatively affected by forest loss.

## Methods

### *Study area*

The study was carried out in the Bodoquena Plateau region, southwest region of the state of Mato Grosso do Sul, specifically in the area covered by the Long-Term Ecological Research PELD - Planalto da Bodoquena. The region has forested and well-preserved environments, such as the Serra da Bodoquena National Park and also fragmented areas, demonstrating landscape complexity and environmental heterogeneity (Boggiane, 1999; ICMBio, 2013). The vegetation in the region is composed of remnants of the Atlantic Forest, and mostly from the Cerrado biome (Brazilian savanna).

Brazilian Cerrado is characterized by a high level of endemism, with a high number of species (Klink & Machado, 2005; Zee-MS, 2009), moreover, is considered a priority area for conservation by the Brazilian Ministry of Environment (Brasil, 2016) (Figure 1). The region has been threatened by anthropogenic land use changes on a large scale, especially with the expansion of agriculture and livestock (Rausch et al., 2019; Strassburg et al., 2017).

The regional climate is tropical, determined as Aw on Köppen-Geiger classification, with wet summers and dry winters (Kottek et al., 2006). The average annual precipitation varies from 1,400 to 1,600 mm, presenting two distinct periods: rainy (October to March) and dry (April to September). The annual average temperatures are between 22 ° and 26 °C, with the relative humidity of the air reaching a maximum of 80% (Baptista-Maria et al., 2009).

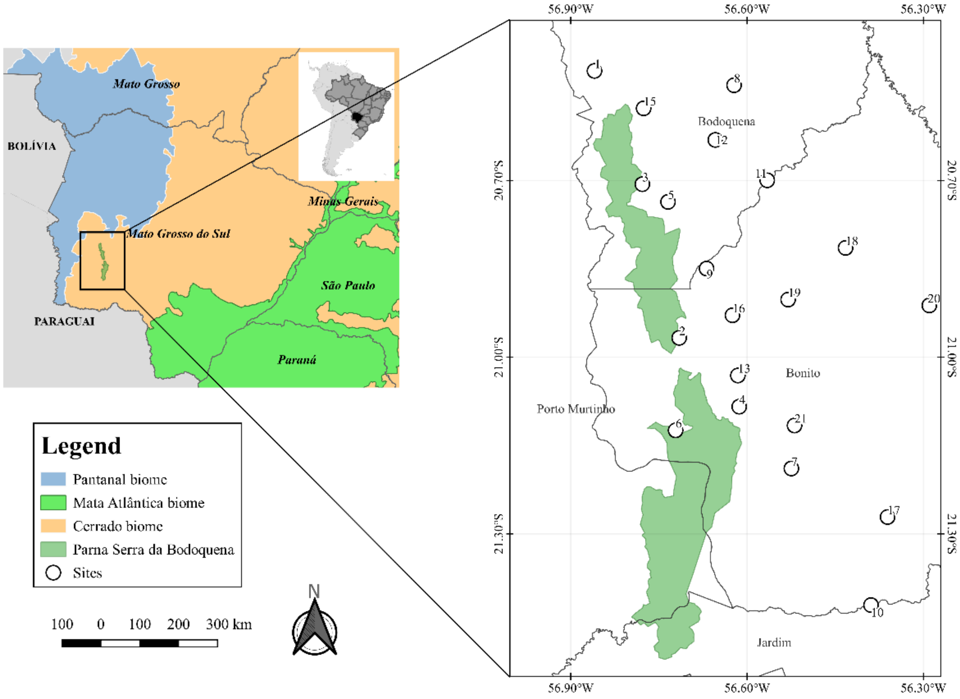


Figure 1. Spatial distribution of the 21 collection sites around forest gradient in Bodoquena Plateau, Mato Grosso do Sul.

### *Mosquito collection*

Mosquitoes were collected in 21 landscapes, using two collection techniques (Figure 1). CDC light traps (one installed in the canopy of a tree and the other near the ground) and one Shannon light traps were installed simultaneously within the forest in each landscape, approximately 200 m away from each other. Mosquitoes were collected between February and July 2019, with a Castro manual suction and tubes impregnated with ethyl acetate from 4:00 pm to 10:00 pm, totaling 126 hours. All collections were performed with personal protective equipment, minimizing contact between mosquitoes and collectors.

The mosquitoes were sent for identification at the Laboratório de Malária e Dengue of the Instituto Nacional de Pesquisas da Amazônia (INPA) and will be deposited in the entomological collection of the Universidade Federal de Mato Grosso do Sul (UFMS) and the Laboratório de Malária e Dengue (INPA). Collections were authorized under SISBIO permit 58866 and mosquitoes were identified using specialized dichotomous Keys (Consoli & Lourenço de Oliveira, 1994; Forattini, 2002).

### *Landscape analysis*

We defined circular concentric buffers with a radius of 500m around each sampling point to estimate the percentage of native vegetation. This buffer represents an approximate area of dispersion movement per day for some genera (e.g. *Anophele*s, *Aedes*, *Culex*, *Haemagogus*, *Sabethes*) (Forattini et al., 1990; Service, 1997). To classify land use, we used 2019 images from the Sentinel-2 Level-1C sensor with a 10-m spatial resolution (ESA, 2015). After processing the images, bands 3, 4 and 8 (green, red and near infrared respectively) were merged to perform a semi-automatic classification in Quantun GIS version 3.4.13 - Madeira, using the Plugin SCP (Semi automatic classification plugin) version 6.4.0 Greenbelt. The classification resulted in binary data (e.g. forest or non-forest) and classification accuracy was tested via the SCP Plugin in Quantum GIS using Google Earth images as references (Supplementary Table S1).

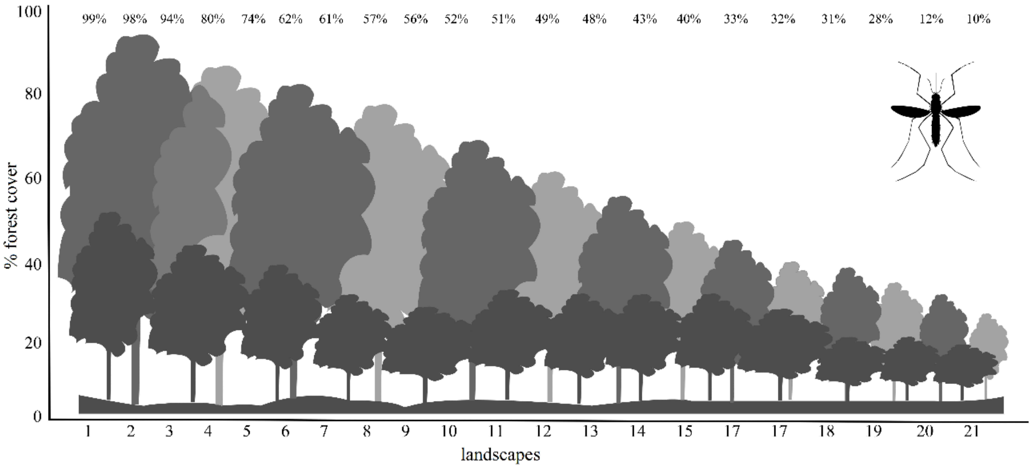


Figure 2. Forest cover gradient ranging from 99 to 10% around 21 points in the Bodoquena Plateau, Mato Grosso do Sul, Brazil.

### *Abiotic variables*

To characterize the areas during each sampling, we measured relative humidity and air temperature using a digital thermo-hygrometer. In addition, we also measure the distance (meters) between the traps and the nearest water body. Daily precipitation amounts (mm) were obtained from the Bonito and Bodoquena automatic meteorological station in the meteorological database for teaching and research (BDMEP) of the National Institute of Meteorology (INMET, 2021).

### *Data analyses*

Canonical correspondence analysis (CCA) was performed using the vegan package (Oksanen et al., 2011) as an exploratory analysis to elucidate the relationship of environmental variables (forest cover, distance of water body and air temperature) and the abundance of mosquito species.

To test our hypothesis, we performed a generalized linear model (GLM with Poisson distribution), a segmented linear regression model (piecewise), and a null model. The effects of the predictor variables (forest cover gradient) on species richness and abundance of the species were accessed through a GLM, assuming a linear relationship. We used segmented regression analysis to identify possible species thresholds along a gradient of loss of native forest cover. The analysis divides explanatory variables into two or more linear regressions, seeking to locate the points where there is a relationship of linear changes. The identification of thresholds (breakpoints) is estimated using different starting points and identifying regressions with higher R2 values (Muggeo, 2003), and the null model represent absence of effects.

To compare the best model for each species, we use the Akaike Information Criterion corrected - AICc for small sample sizes. We considered models with ΔAICc <2 having the strongest empirical support (Burnham & Anderson, 2002). The models were generated using specimens with identification at the species level, and for all models, we used the native forest cover (500m radius) as a predictor variable. Analyses were conducted using the packages “bbmle” (Bolker, 2016), and “segmented” (Muggeo, 2008). All plots were made with package “ggplot2” (Wickham, 2016) in R (R Development Core Team, 2020)

## Results

We registered a total of 6,910 mosquito specimens, belonging to 13 mosquito genera, representing 33 species. The genus *Ochlerotatus* presented the highest number of specimes (4,447) accounted seven species, followed by *Chagasia* (636) with a single species, and *Anopheles* (517) with seven species. The genera *Culex* (361), *Psorophora* (311) and *Haemagogus* (290), with three, four and two species respectively. *Oc.*

*scapularis* was the most abundant and frequently captured species in all landscapes (3,637 individuals, representing 52% of the specimens identified). Then the species *Oc. fulvus* (724), *Ch. bonneae* (636), *Cx.* sp. (348) and *An. triannulatus* with 310 especimens (Table 1, Supplementary Figure S1 and Table S1).

Table 1. Mosquito species collected across a forest cover gradient in Bodoquena Plateau, Mato Grosso do Sul, Brazil.

| Mosquito species | Abundance | % |
|---|---|---|
| *Aedeomyia (Aed.) squamipennis* (Lynch Arribalzaga, 1878) | 1 | 0.01 |
| *Aedes (Stg.) aegypti* (Linnaeus, 1762)* | 3 | 0.04 |
| *Anopheles (Nys.) albitarsis* Lynch Arribálzaga, 1878 | 1 | 0.01 |
| *Anopheles (Nys.) benarrochi* Gabaldon, Cova-García & Lopez, 1941 | 2 | 0.02 |
| *Anopheles (Nys.) darlingi* Root, 1926* | 216 | 3.12 |
| *Anopheles (Nys.) evansae* (Brèthes, 1926) | 9 | 0.13 |
| *Anopheles (Nys.) rangeli* Gabaldon, Cova García & Lopez, 1940 | 4 | 0.05 |
| *Anopheles (Nys.) triannulatus* (Neiva & Pinto, 1922) | 310 | 4.48 |
| *Anopheles* sp. | 29 | 0.41 |
| *Chagasia bonneae* Root, 1927 | 636 | 9.20 |
| *Coquillettidia (Rhy.) albicosta* (Peryassú, 1908) | 1 | 0.01 |
| *Culex (Cx.) quinquefasciatus* Say, 1823* | 10 | 0.14 |
| *Culex (Cx.)* sp. | 3 | 0.04 |
| *Culex (Mel.)* sp. | 348 | 5.03 |
| *Haemagogus (Hag.) janthinomys* Dyar, 1921* | 48 | 0.69 |
| *Haemagogus (Con.) leucocelaenus* (Dyar & Shannon, 1924)* | 242 | 3.50 |
| *Mansonia (Man.) humeralis* Dyar & Knab, 1916 | 3 | 0.04 |
| *Mansonia (Man.) titillans* (Walker, 1848)* | 147 | 2.12 |
| *Mansonia (Man.)* sp. | 42 | 0.60 |
| *Ochlerotatus (How.) fulvithorax*Lutz, 1904 | 57 | 0.82 |
| *Ochlerotatus (Och.) fulvus* (Wiedemann, 1828) | 724 | 10.47 |
| Ochlerotatus (Och.) *scapularis* Rondani, 1848* | 3637 | 52.63 |
| *Ochlerotatus (Och.) serratus* (Theobald, 1901)* | 8 | 0.11 |
| *Ochlerotatus (Och.)* sp. | 21 | 0.30 |
| *Psorophora (Jan.) albigenu* (Peryassú, 1908) | 301 | 4.35 |
| *Psorophora (Gra.) cingulata* (Fabricius, 1805) | 3 | 0.04 |
| *Psorophora (Jan.) ferox* (von Humboldt, 1819)* | 5 | 0.07 |
| *Psorophora* sp. | 2 | 0.02 |
| *Sabethes (Sab.) belisarioi* Neiva, 1908* | 1 | 0.01 |
| *Sabethes (Sob.) glaucodaemon* (Dyar & Shannon, 1925) | 9 | 0.13 |

| | | |
|---|---|---|
| *Uranotaenia* sp. | 1 | 0.01 |
| *Wyeomyia (Den.) aporonama* Dyar & Knab, 1906 | 85 | 0.23 |
| *Wyeomyia (Den.)* sp. | 1 | 0.01 |
| Total | 6910 | 100 |

* Vector species

The relative humidity of the air in the landscapes ranged from 47.9 to 73.7% (mean= 64.1 ± 6.4% standard deviation), and the air temperature ranged from 24.0 to 27.9°C, with mean of 26.0 ± 0.9°C standard deviation. The mean amount of rainfall was 7.7 mm ± 12.3 mm standard deviation, ranged from 0.0 to 47.6 mm (Supplementary Table S1).

The first axis of CCA captured 80.0% of constrained inertia and the second axis captured 15.0%, and the two generated axes explained 95.0%. The CCA ordination shows a clear separation between three groups, showing greater association of species in group 1 with the forest variable. In addition, the species in group 3 were more related to the variables of temperature and distance from the water body, however, the group of species 3 were not associated with the environmental variables addressed (Figura 3).

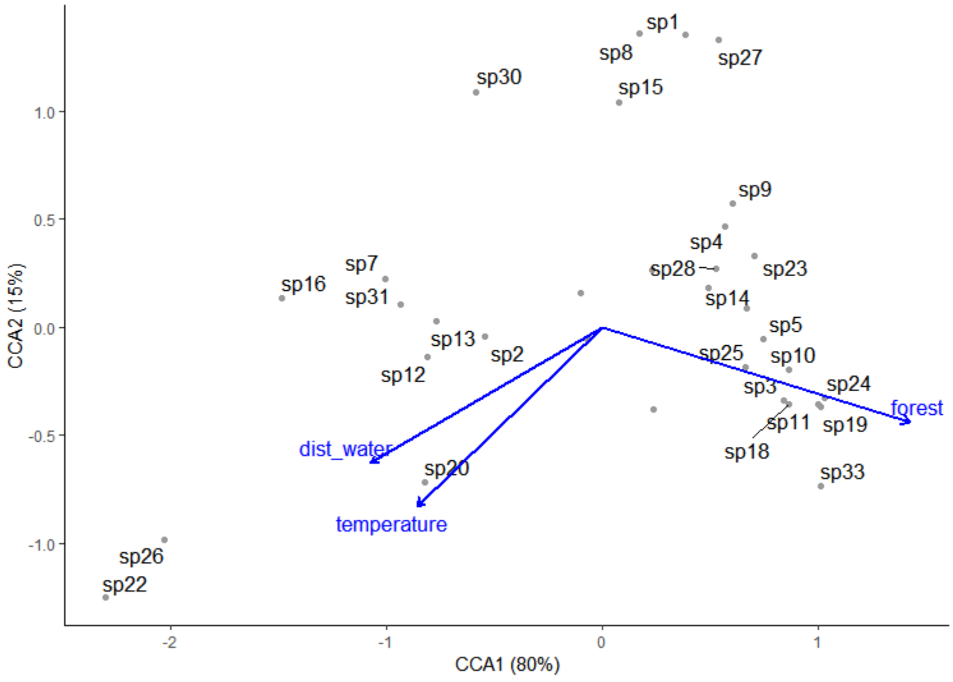


Figure 3. Ordering diagram of the canonical correlation analysis (CCA) of the relationship between the environmental factors (air temperature, % forest cover, and distance of water), and abundance of mosquitoes (sp1: *Oc. serratus*, sp2: *Oc. scapularis*, sp3: *Oc. fulvus*, sp4: *Oc. fulvithorax*, sp5: *Oc*. sp, sp6: *Ps. albigenu*, sp7: *Ps. cingulata*, sp8: *Ps. ferox*, sp9: *Ps*. sp, sp10: *Hg. leucocelaenus*, sp11: *Hg. janthinomys*, sp12: *Ma. titillans*, sp13: *Ma. humeralis*, sp14: *Ma*. sp, sp15: *An. darlingi*, sp16: *An. albitarsis*, sp17: *An. benarrochi*, sp18: *An. triannulatus*, sp19: *An. rangeli*, sp20: *An. evansae*, sp21: *An*. sp, sp22: *Ae. aegypti*, sp23: *Ch. bonnae*, sp24: *Wy. aporonama*, sp25: *Wy*. sp, sp26: *Cx. quinquefasciatus*, sp27: *Cx*. (*Cx.*) sp, sp28: *Cx*. (*Mel.*) sp, sp29: *Cq. albicosta*, sp30: *Ad. squamipennis*, sp31: *Ur*. sp, sp32: *Sa. belisarioi*, sp33: *Sa. glaucodaemon*).

The best fit model for total abundance, vectors and non-vector species was the generalized linear model - GLM. The culicid abundance was positively related to forest cover in the studied landscapes. On the other hand, vector species were negatively associated with forest cover, and the non-vector species was positively related to forest cover. The variation in species abundance was highly explained by the percentage of forest cover (Figure 4, Table 2).

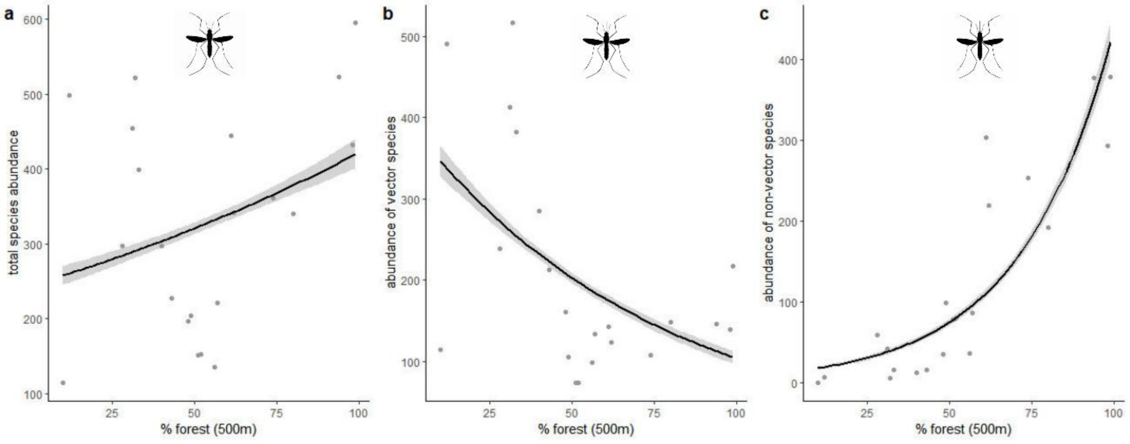


Figure 4. Relationships between mosquito abundance (a), vector species (b), non-vector especies (c) and percentage of forest cover at a radius of 500m, modelled by generalized linear models. Gray shades represent the confidence interval (95%) for the generalized linear model fitted.

The best fit model for a group of species was the piecewise regression model. We detected thresholds for 14 species along the gradients of forest cover. The *Ma. titillans* change threshold was 81% of forest cover loss. The threshold for *Oc. scapularis* and *Oc. fulvus* was 12% of forest cover. The thresholds for *Ch. bonneae*, *Hg. leucocelaenus*, *Oc. fulvithorax*, *An. darlingi* were between 61 - 62%, and to the *Ps. albigenu*, *Hg. janthinomys*, *An. triannulatus*, *Wy. poronama*, *Sa. glaucodaemon*, we observe a threshold between 48 - 54% of native forest cover. For *An. albitarsis*, a threshold at the value of 30% forest cover was found, and for *Cx. quinquefasciatus* and *Ae. aegypti*, the threshold was around 12% of forest cover. The null model was the best fit for other species (Table 2, Figure 5).

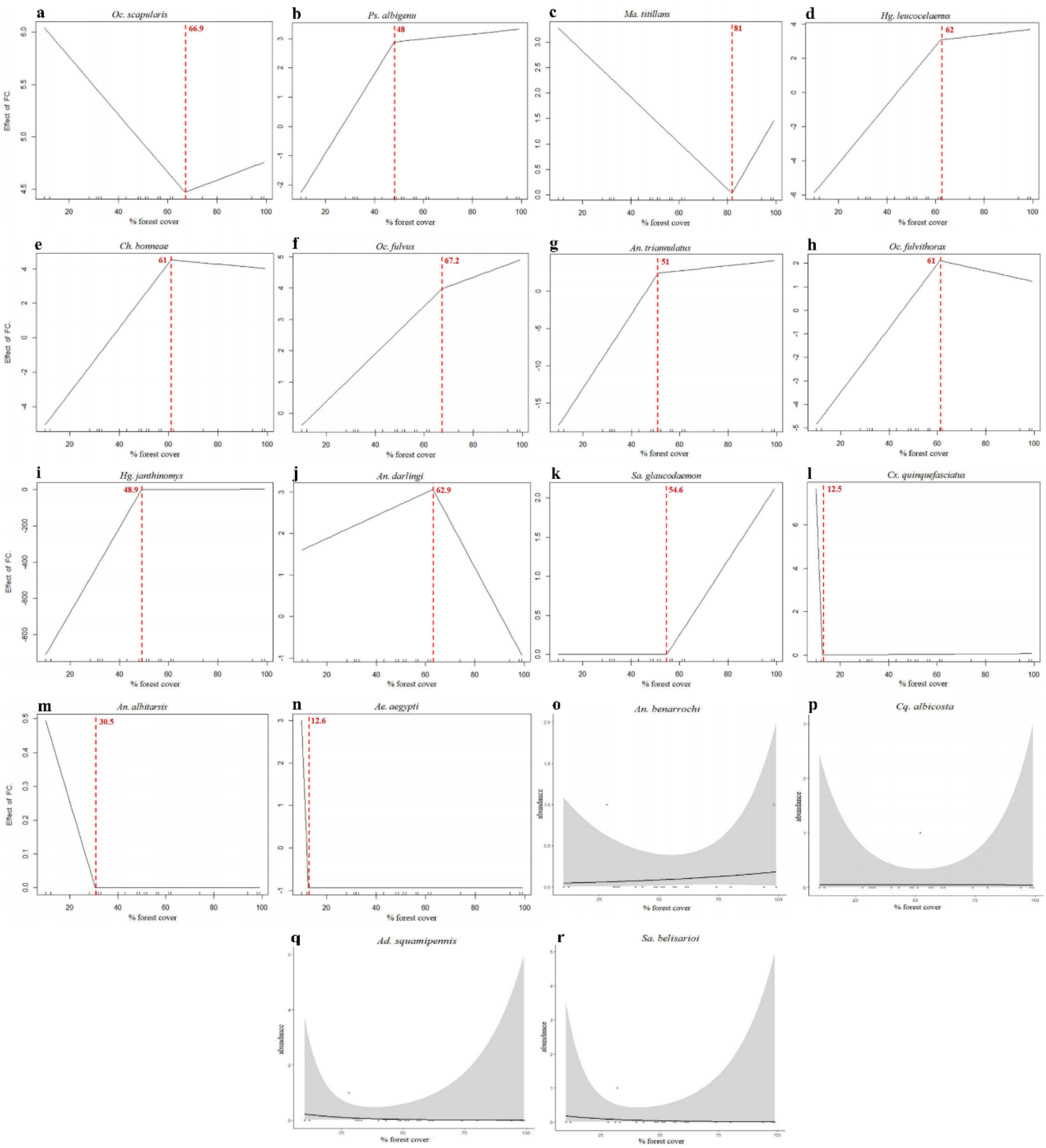


Figure 5. Relationships between the abundance of each mosquito specie and the percentage of native forest cover across landscapes gradientes in Bodoquena Plateau, Mato Grosso do Sul. The dashed red line indicates the threshold of forest cover of the species. Gray shades represent the confidence interval (95%) for the generalized linear model fitted. Letters (a) to (n) are the best fit for piecewise-regression model, and letters (m) to (r) are the best fit for generalized linear model.

Table 2. Statistical models proposed for the total abundance of mosquitoes, vector species, non-vector species, and each Culicidae specie across gradients of forest cover loss in Bodoquena Plateau, Mato Grosso do Sul. AICc: Akaike Information Criterion corrected; dAICc: difference in AICc between the best model (best ranked models AICc < 2.0); df: degrees of freedom; weight: is information criteria weight; intercept: both regressions; slope 1: corresponds to the slope of regression before thresholds; slope 2: slope of segmented regression after the thresholds; threshold: corresponds to estimated breakpoint; The best fit model is indicated in bold. NA is the species that are negatively affected by forest loss.

| Models | AICc | dAICc | df | Weight | Intercept (se) | Slope 1 (se) | Slope 2 (se) | Threshold | NA |
|---|---|---|---|---|---|---|---|---|---|
| Species abundance | | | | | | | | | |
| **GLM** | **1002.5** | **0.0** | **2** | **1** | **6.13 (0.04)** | **–0.012 (0.001)** | – | – | YES |
| PIECEWISE | 1369.0 | 366.5 | 4 | <0.001 | 5.49 (0.02) | 0.005 (0.00) | 0.03 (0.001) | 52 | |
| NULL | 1498.2 | 495.7 | 1 | <0.001 | 5.79 (0.01) | – | – | – | |
| Vector species | | | | | | | | | |
| **GLM** | **1061.8** | **0.0** | **2** | **1** | **6.30 (0.03)** | **–0.02 (0.00)** | – | – | NO |
| PIECEWISE | 1261.3 | 199.5 | 4 | <0.001 | 5.98 (0.03) | –0.01 (0.00) | 0.03 (0.003) | 69.1 | |
| NULL | 1699.6 | 637.8 | 1 | <0.001 | 5.32 (0.01) | – | – | – | |
| Non-vector species | | | | | | | | | |
| **GLM** | **538.5** | **0.0** | **2** | **1** | **0.45 (0.18)** | **0.07 (0.003)** | – | – | YES |
| PIECEWISE | 787.1 | 248.6 | 4 | <0.001 | 2.56 (0.06) | 0.35 (0.00) | –0.06 (0.004) | 62.0 | |
| NULL | 2776.4 | 2237.9 | 1 | <0.001 | 4.81 (0.01) | – | – | – | |
| *Oc. scapularis* | | | | | | | | | |
| GLM | 1367.2 | 145.6 | 2 | <0.001 | 6.03 (0.03) | –0.01 (0.00) | – | – | NO |
| **PIECEWISE** | **1221.6** | **0.0** | **4** | **1** | **6.31 (0.04)** | **–0.02 (0.001)** | **0.03 (0.004)** | **66.9** | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| NULL | 2046.3 | 824.7 | 1 | <0.001 | 5.15 (0.01) | – | – | – | |
| *Ps. albigenu* | | | | | | | | | |
| GLM | 207.1 | 61.6 | 2 | <0.001 | 1.21 (0.16) | 0.02 (0.002) | – | – | YES |
| **PIECEWISE** | **145.5** | **0.0** | **4** | **1** | **–3.61 (1.05)** | **0.13 (0.02)** | **–0.12 (0.02)** | **48.0** | |
| NULL | 316.6 | 171.2 | 1 | <0.001 | 2.66 (0.05) | – | – | – | |
| *Ma. titillans* | | | | | | | | | |
| GLM | 163.6 | 11.2 | 2 | 0.003 | 3.42 (0.16) | –0.03 (0.004) | – | – | NO |
| **PIECEWISE** | **152.3** | **0.0** | **4** | **0.996** | **3.72 (0.16)** | **–0.04 (0.004)** | **0.12 (0.14)** | **81.8** | |
| NULL | 244.1 | 91.7 | 1 | <0.001 | 1.94 (0.08) | – | – | – | |
| *Hg. leucocelaenus* | | | | | | | | | |
| GLM | 153.1 | 57.6 | 2 | <0.001 | –0.75 (0.25) | 0.04 (0.003) | – | – | YES |
| **PIECEWISE** | **95.4** | **0.0** | **4** | **1** | **–7.58 (1.35)** | **0.17 (0.02)** | **–0.15 (0.02)** | **62.0** | |
| NULL | 459.7 | 364.3 | 1 | <0.001 | 2.44 (0.06) | – | – | – | |
| *Ch. bonneae* | | | | | | | | | |
| GLM | 820.6 | 398.4 | 2 | <0.001 | 1.41 (0.12) | 0.03 (0.001) | – | – | YES |
| **PIECEWISE** | **422.2** | **0.0** | **4** | **1** | **–6.94 (0.79)** | **0.18 (0.01)** | **–0.20 (0.01)** | **61.0** | |
| NULL | 1221.3 | 799.1 | 1 | <0.001 | 3.41 (0.03) | – | – | – | |
| *Oc. fulvus* | | | | | | | | | |
| GLM | 285.3 | 24.6 | 2 | <0.001 | 0.39 (0.14) | 0.04 (0.001) | – | – | YES |
| **PIECEWISE** | **260.7** | **0.0** | **4** | **1** | **–1.13 (0.41)** | **0.07 (0.007)** | **–0.04 (0.008)** | **67.2** | |
| NULL | 1184.4 | 923.7 | 1 | <0.001 | 3.54 (0.03) | – | – | – | |
| *An. triannulatus* | | | | | | | | | |
| GLM | 173.6 | 53.5 | 2 | <0.001 | –0.62 (0.22) | 0.04 (0.002) | – | – | YES |
| **PIECEWISE** | **120.1** | **0.0** | **4** | **1** | **–22.92 (7.79)** | **0.49 (0.15)** | **–0.46 (0.15)** | **51.0** | |
| NULL | 586.8 | 466.7 | 1 | <0.001 | 2.69 (0.05) | – | – | – | |
| *Oc. fulvithorax* | | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| GLM | 117.4 | 29.3 | 2 | <0.001 | –0.45 (0.38) | 0.02 (0.005) | – | – | YES |
| **PIECEWISE** | **88.21** | **0.0** | **4** | **1** | **–6.20 (2.06)** | **0.13 (0.03)** | **–0.15 (0.04)** | **61.0** | |
| NULL | 136.3 | 48.2 | 1 | <0.001 | 0.99 (0.13) | – | – | – | |
| *Hg. janthinomys* | | | | | | | | | |
| GLM | 62.2 | 1.1 | 2 | 0.37 | –3.18 (0.66) | 0.05 (0.007) | – | – | YES |
| **PIECEWISE** | **61.1** | **0.0** | **4** | **0.63** | **–1143.47 (8554.80)** | **23.53 (258.53)** | **–23.31 (258.53)** | **48.9** | |
| NULL | 140.8 | 79.7 | 1 | <0.001 | 0.82 (0.14) | – | – | – | |
| *An. darlingi* | | | | | | | | | |
| GLM | 471.2 | 86.0 | 2 | <0.001 | 2.66 (2.15) | –0.006 (0.002) | – | – | YES |
| **PIECEWISE** | **385.2** | **0.0** | **4** | **1** | **1.32 (0.26)** | **0.02 (0.005)** | **–0.13 (0.25)** | **62.9** | |
| NULL | 474.4 | 89.2 | 1 | <0.001 | 2.33 (0.06) | – | – | – | |
| *Wy. poronama* | | | | | | | | | |
| GLM | 121.9 | 10.2 | 3 | 0.006 | –7.57 (1.99) | 0.21 (0.03) | – | – | YES |
| **PIECEWISE** | **111.7** | **0.0** | **5** | **0.993** | **0.000 (0.23)** | **–0.000 (0.006)** | **0.03 (0.008)** | **50.2** | |
| NULL | 245.7 | 134.0 | 1 | <0.001 | 1.39 (0.10) | – | – | – | |
| *Sa. glaucodaemon* | | | | | | | | | |
| GLM | 43.7 | 3.8 | 3 | 0.099 | –0.90 (0.30) | 0.02 (0.005) | – | – | YES |
| **PIECEWISE** | **39.9** | **0.0** | **2** | **0.647** | **0.000 (0.04)** | **–0.000 (0.001)** | **0.004 (0.001)** | **54.6** | |
| NULL | 41.8 | 1.9 | 1 | 0.255 | –0.84 (0.33) | – | – | – | |
| *An. rangeli* | | | | | | | | | |
| GLM | 31.8 | 7 | 3 | 0.029 | –0.33 (0.23) | 0.01 (0.004) | – | – | YES |
| PIECEWISE | 36.8 | 12 | 5 | 0.002 | 0.000 (0.04) | –0.000 (0.001) | 0.001 (0.001) | 48.6 | |
| **NULL** | **24.8** | **0** | **1** | **0.968** | **–1.65 (0.50)** | – | – | – | |
| *An. evansae* | | | | | | | | | |
| GLM | 73.3 | 25.5 | 3 | <0.001 | 0.83 (0.62) | –0.007 (0.01) | – | – | NO |
| PIECEWISE | 77.8 | 30.1 | 5 | <0.001 | –0.69 (1.37) | 0.05 (0.05) | –0.06 (0.05) | 33.0 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **NULL** | **47.8** | **0.0** | **1** | **1** | **–0.84 (0.33)** | – | – | – | |
| *Oc. serratus* | | | | | | | | | |
| GLM | 83.8 | 33.2 | 3 | <0.001 | 0.30 (0.80) | 0.001 (0.01) | – | – | YES |
| PIECEWISE | 89.2 | 38.6 | 5 | <0.001 | –0.70 (1.46) | 0.03 (0.04) | –0.04 (0.05) | 49.0 | |
| **NULL** | **50.7** | **0.0** | **1** | **1** | **–0.96 (0.35)** | – | – | – | |
| *Ps. cingulata* | | | | | | | | | |
| GLM | 32.7 | 11.5 | 3 | 0.003 | 0.45 (0.23) | –0.005 (0.004) | – | – | NO |
| PIECEWISE | 33.8 | 12.6 | 5 | 0.001 | 1.46 (0.57) | –0.04 (0.03) | 0.04 (0.03) | 29.2 | |
| **NULL** | **21.2** | **0.0** | **1** | **0.994** | **–1.94 (0.57)** | – | – | – | |
| *Ps. ferox* | | | | | | | | | |
| GLM | 61.0 | 28.1 | 3 | <0.001 | 0.30 (0.46) | –0.001 (0.008) | – | – | YES |
| PIECEWISE | 65.0 | 32.1 | 5 | <0.001 | –0.52 (0.82) | 0.02 (0.02) | –0.03 (0.02) | 49.0 | |
| **NULL** | **32.9** | **0.0** | **1** | **1** | **–1.43 (0.44)** | – | – | – | |
| *Ma. humeralis* | | | | | | | | | |
| GLM | 33.6 | 12.4 | 3 | 0.002 | 0.38 (0.24) | –0.004 (0.004) | – | – | NO |
| PIECEWISE | 33.6 | 12.4 | 5 | 0.002 | 1.46 (0.56) | –0.04 (0.03) | 0.04 (0.03) | 30.5 | |
| **NULL** | **21.2** | **0.0** | **1** | **0.996** | **–1.94 (0.57)** | – | – | – | |
| *An. benarrochi* | | | | | | | | | |
| **GLM** | **15.1** | **0.0** | **3** | **0.49** | **0.01 (0.15)** | **0.001 (0.002)** | – | – | NO |
| PIECEWISE | 18.0 | 2.9 | 5 | 0.12 | 0.18 (0.18) | –0.002 (0.003) | 0.07 (0.07) | 91.2 | |
| NULL | 15.6 | 0.5 | 1 | 0.39 | –2.35 (0.70) | – | – | – | |
| *Cx. quinquefasciatus* | | | | | | | | | |
| GLM | 85.9 | 36.3 | 3 | <0.001 | 2.04 (0.84) | –0.02 (0.01) | – | – | NO |
| **PIECEWISE** | **49.6** | **0.0** | **5** | **0.993** | **37.80 (4.90)** | **–3.01 (0.44)** | **3.01 (0.44)** | **12.5** | |
| NULL | 59.6 | 10.0 | 1 | 0.006 | –0.74 (0.31) | – | – | – | |
| *An. albitarsis* | | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| GLM | –1.0 | 1.7 | 3 | 0.298 | 0.21 (0.10) | –0.003 (0.001) | – | – | NO |
| **PIECEWISE** | **–2.7** | **0.0** | **5** | **0.701** | **0.73 (0.23)** | **–0.02 (0.01)** | **0.02 (0.01)** | **30.5** | |
| NULL | 10.2 | 13.0 | 1 | 0.001 | –3.04 (1.00) | – | – | – | |
| *Ae. aegypti* | | | | | | | | | |
| GLM | 44.8 | 1233.9 | 3 | <0.001 | 0.66 (0.31) | –0.009 (0.005) | – | – | NO |
| **PIECEWISE** | **–1184.1** | **0.0** | **5** | **1** | **1.8 (0.000)** | **–0.15 (0.000)** | **0.15 (0.000)** | **12.6** | |
| NULL | 23.4 | 1212.6 | 1 | <0.001 | –1.94 (0.57) | – | – | – | |
| *Cq. albicosta* | | | | | | | | | |
| **GLM** | **2.0** | **0.0** | **3** | **0.903** | **0.005 (0.01)** | **–0.000 (0.000)** | – | – | YES |
| PIECEWISE | 6.8 | 4.8 | 5 | 0.82 | –0.11 (0.18) | 0.004 (0.004) | –0.008 (0.006) | 52.0 | |
| NULL | 10.2 | 8.3 | 1 | 0.015 | –3.04 (1.00) | – | – | – | |
| *Ad. squamipennis* | | | | | | | | | |
| **GLM** | **0.9** | **0.0** | **3** | **0.942** | **0.14 (0.11)** | **–0.001 (0.001)** | – | – | NO |
| PIECEWISE | 7.2 | 6.2 | 5 | 0.041 | 0.21 (0.17) | –0.003 (0.004) | 0.003 (0.007) | 58.4 | |
| NULL | 10.2 | 9.3 | 1 | 0.008 | –3.04 (1.00) | – | – | – | |
| *Sa. belisarioi* | | | | | | | | | |
| **GLM** | **2.0** | **0.0** | **3** | **0.905** | **0.05 (0.11)** | **–0.000 (0.001)** | – | – | YES |
| PIECEWISE | 6.8 | 4.8 | 5 | 0.081 | –0.10 (0.19) | 0.004 (0.005) | –0.008 (0.006) | 51.0 | |
| NULL | 10.2 | 8.3 | 1 | 0.015 | –3.04 (1.00) | – | – | – | |

## Discussion

We identified a positive effect of the amount of forest cover on the total abundance of mosquitoes in the landscapes. In addition, non-vector species are more susceptible to landscapes with low forest cover, negatively affecting the abundance of these species. On the other hand, the high abundance of vector species was associated with a low percentage of native forest cover. The thresholds values ranged from 12.5 to 81% of forest cover and presented different values for the 14 species. When looking at the sum of the species, we detect a clear pattern of forest cover by sylvatic species (around 60%) and low thresholds values for urban and anthropophilic species (aproximadely 12%).

Forest cover has been approached as an important predictor for the composition, distribution and abundance of several species, especially mosquitoes that are vectors of diseases (Moyes et al., 2016; Chaves et al., 2021, see also Chapter 2). Mosquitoes have been used as important bioindicators of environmental degradation (Dorvillé, 1996) helping to assess the degree of environmental changes in a given area (Alencar et al., 2021), responding to changes in the landscape with drastic changes in its density or the extinction of certain species (Medeiros-Sousa et al., 2015; Chaves et al., 2016). Studies have shown positive and negative effects of forest cover on the occurrence of key species for Public Health, such as *An. darlingi*, *Cx. quinquefasciatus*, *Ae. aegypti*, *Oc. scapularis* (Wilke et al., 2017; Chaves et al., 2021). In our study we added evidences that forest cover plays an important role for the 25 species found along the forest cover gradient, showing a strong relationship of sylvatic vector species in landscapes with high values of forest cover (e.g. *Hg. leucocelaenu*s, *Hg. janthinomys*, *An. darlingi*), and species with greater adaptability to urban regions and anthropogenic areas with less native forest cover (e.g. *Oc. scapularis*, *Ae. aegypti*, *Ma. titillans*, *Cx. quinquefasciatus*).

These results show that forest cover is an important modulator of the total abundance of mosquitoes. Moreover, the deconstruction of the community in vector and non-vector species helps us to reveal important patterns usually masked when pooling all species (Marquet et al., 2004).

We identified 28 species of mosquitoes, with the occurrence of ten vector species of medical importance. Among them, *An. darlingi* (Malaria), *Ae. aegypti* (Dengue, Zika, Chikungunya), *Cx. quinquefasciatus* (Filariose, Oropouche fever), *Hg. leucocelaenus* (Sylvatic Yellow fever), *Hg. janthinomys* (Sylvatic Yellow fever), *Ma. titillans* (Venezuelan Equine Encephalitis virus), *Oc. scapularis* (Rocio Encephalitis virus, Dirofilariose, Filariose), *Oc. serratus* (Oropouche fever, secondary vector of Sylvatic Yellow fever), *Ps. ferox* (Rocio virus), *Sa. belisarioi* (Sylvatic Yellow fever), are important species with vector competence in Brazil (Forattini, 2002; Cardoso et al., 2010, Leal-Santos et al., 2017; Tadei et al., 2017; Arcos et al., 2018). Although these species have already been reported in forest remnants and riparian forest in Mato Grosso do Sul (Almeida et al., 2016), the occurrence of them throughout the landscapes in the Bodoquena Plateau is relevant from an epidemiological point of view, beucause this region has a high movement of people, including from other countries, due to touristic activities.

In the present study, the species richness decreased with the loss of forest cover along the gradient, enabling the emergence of potential vector species. This relationship can also be seen between a strong link between the loss of culicid species and the increased risk of pathogen transmission in urban landscapes (Medeiros-Sousa et al., 2017). Moreover, the urbanization process positively affects the abundance of mosquitoes of medical importance (Wilke et al., 2017), and changes in land use and anthropic activities are being incriminated as potential drivers in the emergence of virus and arboviruses (Jones et al., 2008; Donalisio et al., 2017). The response that some species have to the loss of forest cover may be related to the biological and ecological characteristics of mosquitoes, such as the preference of blood meal (zoophilic and anthropophilic), availability of aquatic habitat for oviposition, presence of animals and humans and microclimate factors (Barros e al., 2010; Burkett-Cadena & Vittor, 2018).

Most studies on mosquitoes have detected the effect of forest cover on mosquito biodiversity, with an indication of potential impact on the abundance of vector species and the risk of disease emergence (Ferraguti et al., 2016; Tadei et al., 2017; Vieira et al., 2021). Studies on tropical biodiversity have shown that the loss of forests can cause non-linear resposes on different groups, such as mammals, fish, anphibians, aquatic insects (Ochoa-Quintero et al., 2015; Rodrigues et al., 2016; Dala-Corte et al., 2020; Verga et al., 2021; Valente-Neto et al., 2021), however, for mosquitoes few evidences exist if they responsed in a non-linear way to gradients of native vegetation (Wilke et al., 2017; Câmara et al., 2020). Under this perspective, our study advances towards detecting thresholds for vector and non-vector species, and their relationship with the loss of forest cover. We found that about 46% of the species had thresholds between 81 and 61%, 40% of the species had between 54 and 30%, and 13% had low thresholds around 12% of forest cover. In this sense, the vector species showed forest cover thresholds between moderate and low, demonstrating their versatility and adaptation in altered areas, and the identification of the effects of land use on the mosquito community and its thresholds, is linked with potential implications for the emergence of arboviruses. For example, the altered environments can force these species to migrate and, consequently, to change hosts and increasing the vectors contact with man and domestic animals (Vinod, 2011).

When the percentage of forest cover exceeds the threshold values, some aquatic and terrestrial organisms (e.g. dragonflies) are affected at the same time (Rodrigues et al., 2016), this effect can be extrapolated to several mosquito species that use both environments in their life cycle, especially sylvatic species. On the other hand, the relationship between land use and habitat loss has been increasing the richness and abundance of arboviral vectors, occurring in landscapes with medium and high degree of anthropization (Vieira et al., 2021). Two urban vector species were considered rare in this study (*Ae. aegypti* and *Cx. quinquefasciatus*) presented thresholds around 12%, being present in landscapes with a high degree of modification, demonstrating their relationship with altered and urban environments. In addition, six vector species were more likely to occur in landscapes with medium or high degree of disturbance, with thresholds ranging from 12 to 60% of forest cover. We emphasize that as deforestation increases in the region, there is a clear loss of species and an increase in the presence of potential disease vectors for animals and humans.

In general, below 48% of forest cover the mosquito biodiversity is negatively affected, in contrast, many vector species end up benefiting from the decrease in native forest cover (e.g. *Oc. scapularis, Ma. titillans, An. darlingi, An. albitarsis, Ae. aegypti, Cx. quinquefasciatus*). This main result has implications for public health because if a landscape will loose more than ~50% of forest cover, it can favor important species that transmit diseases. Furthermore, maintaining the integrity of forest ecosystems, especially with the reduction of deforestation helps (1) in maintaining biodiversity, (2) in the natural balance between wild animals and their own pathogens, (3) decreased contact between vectors and domestic animals and humans, (3) decreased cases of diseases, favoring the maintenance of human health (Keusch et al., 2009; Watson et al., 2018; Ellwanger et al., 2020).

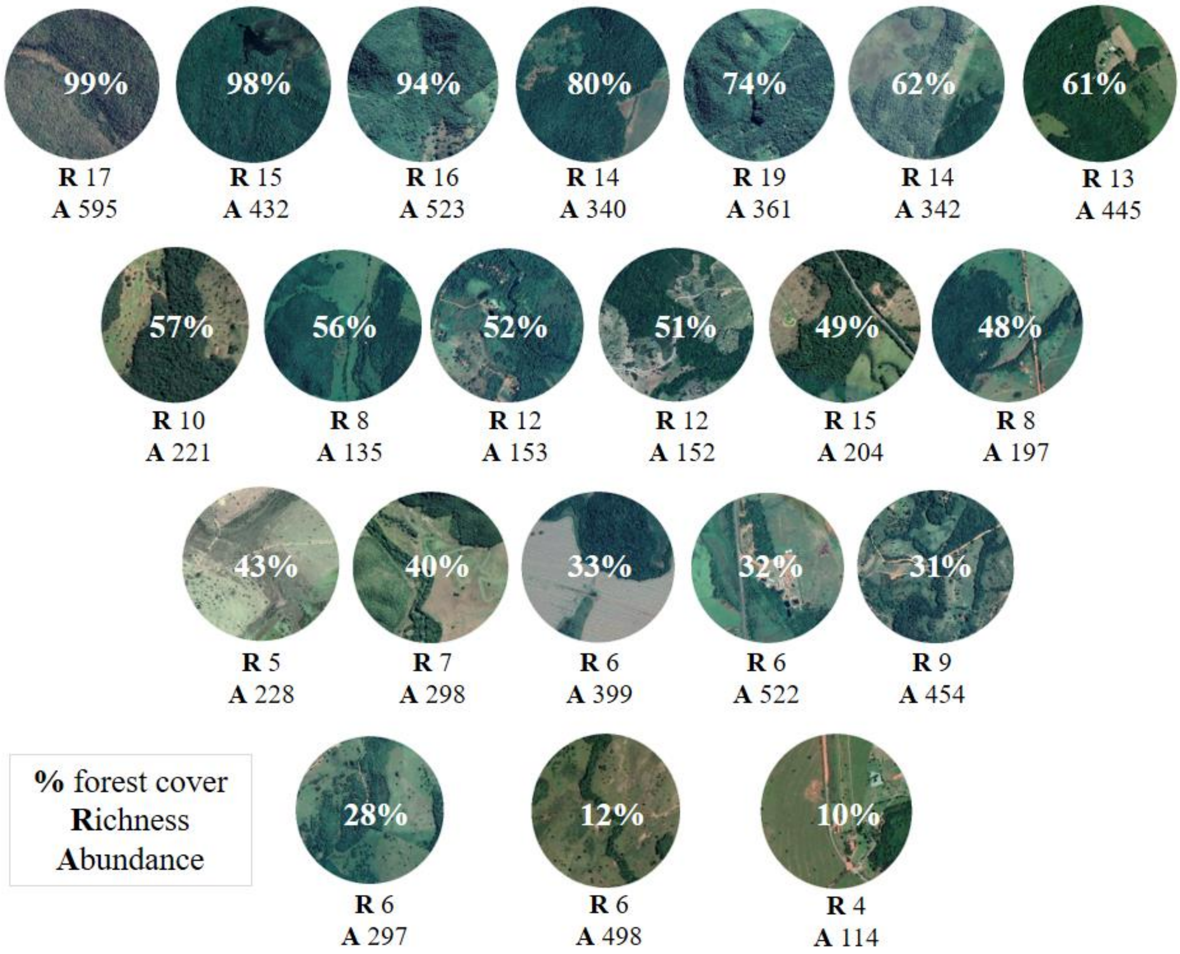
# Supporting Information for Chapter 3

Suppl. Table 1. Distribution of mosquitoes and environmental variables along the forest cover gradients (%) in Bodoquena Plateau, Mato Grosso do Sul, Brazil.

| Species | Landscapes with % forest cover | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | P11 | P12 | P13 | P14 | P15 | P16 | P17 | P18 | P19 | P20 | P21 |
| | 99% | 98% | 94% | 80% | 74% | 62% | 61% | 57% | 56% | 52% | 51% | 49% | 48% | 43% | 40% | 33% | 32% | 31% | 28% | 12% | 10% |
| *Oc. serratus* | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Oc. scapularis* | 138 | 97 | 101 | 115 | 83 | 58 | 87 | 121 | 86 | 42 | 57 | 53 | 146 | 205 | 273 | 366 | 495 | 371 | 187 | 458 | 98 |
| *Oc. fulvus* | 162 | 118 | 99 | 58 | 83 | 46 | 30 | 12 | 20 | 25 | 11 | 35 | 2 | 0 | 0 | 0 | 0 | 23 | 0 | 0 | 0 |
| *Oc. fulvithorax* | 6 | 6 | 2 | 0 | 4 | 12 | 9 | 5 | 3 | 1 | 0 | 8 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Oc. sp* | 5 | 2 | 1 | 3 | 2 | 2 | 0 | 3 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ps. albigenu* | 33 | 18 | 35 | 12 | 29 | 27 | 16 | 20 | 10 | 13 | 31 | 11 | 27 | 5 | 8 | 2 | 0 | 2 | 0 | 2 | 0 |
| *Ps. cingulata* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| *Ps. ferox* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ps. sp* | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Hg. leucocelaenus* | 53 | 29 | 35 | 31 | 21 | 29 | 16 | 11 | 7 | 1 | 2 | 2 | 1 | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 |
| *Hg. janthinomys* | 16 | 10 | 6 | 1 | 2 | 5 | 2 | 0 | 0 | 1 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ma. titillans* | 5 | 3 | 3 | 1 | 2 | 2 | 0 | 2 | 5 | 0 | 2 | 0 | 1 | 7 | 9 | 16 | 19 | 21 | 11 | 33 | 5 |
| *Ma. humeralis* | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| *Ma. sp* | 2 | 3 | 7 | 2 | 14 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| *An. darlingi* | 4 | 0 | 1 | 0 | 0 | 29 | 37 | 0 | 0 | 29 | 8 | 36 | 13 | 0 | 0 | 0 | 0 | 19 | 40 | 0 | 0 |
| *An. albitarsis* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| *An. benarrochi* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| *An. triannulatus* | 65 | 57 | 36 | 21 | 49 | 17 | 22 | 14 | 2 | 9 | 12 | 5 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *An. rangeli* | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *An. evansae* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| *An. sp* | 0 | 0 | 0 | 10 | 3 | 0 | 5 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 4 | 1 | 0 | 0 | 0 | 0 |
| *Ae. aegypti* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| *Ch. bonnae* | 77 | 40 | 82 | 51 | 39 | 44 | 212 | 32 | 0 | 22 | 4 | 15 | 3 | 0 | 3 | 6 | 0 | 6 | 0 | 0 | 0 |
| *Wy. aporonama* | 17 | 14 | 19 | 11 | 11 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Wy. sp* | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Cx. quinquefasciatus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 8 |
| *Cx. (Cx.) sp* | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Cx. (Mel.) sp* | 8 | 32 | 94 | 21 | 13 | 49 | 5 | 0 | 0 | 8 | 21 | 15 | 4 | 10 | 1 | 0 | 4 | 6 | 57 | 0 | 0 |
| *Cq. albicosta* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ad. squamipennis* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| *Ur. sp* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| *Sa. belisarioi* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Sa. glaucodaemon* | 2 | 2 | 1 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Abundance | 595 | 432 | 523 | 340 | 361 | 342 | 445 | 221 | 135 | 153 | 152 | 204 | 197 | 228 | 298 | 399 | 522 | 454 | 297 | 498 | 114 |
| Richness | 17 | 15 | 16 | 14 | 19 | 14 | 13 | 10 | 8 | 12 | 12 | 15 | 8 | 5 | 7 | 6 | 6 | 9 | 6 | 6 | 4 |
| Humidity % | 73.7 | 70.7 | 71.1 | 70.6 | 69.7 | 69.1 | 68.8 | 61.0 | 69.0 | 68.0 | 59.8 | 67.5 | 62.1 | 61.1 | 59.3 | 60.0 | 59.3 | 50.0 | 66.0 | 52.5 | 55.2 |
| Humidity % | 71.9 | 71.1 | 70.2 | 71.0 | 69.6 | 70.2 | 70.1 | 63.0 | 70.0 | 66.9 | 59.9 | 66.2 | 61.8 | 61.6 | 60.1 | 59.8 | 59.0 | 49.1 | 64.0 | 54.0 | 56.0 |
| Humidity % | 72.5 | 71.3 | 70.7 | 71.3 | 70.0 | 70.1 | 69.5 | 64.0 | 71.0 | 67.7 | 60.2 | 65.0 | 62.6 | 61.9 | 58.7 | 59.3 | 60.0 | 47.9 | 65.4 | 55.1 | 55.8 |
| Temperature °C | 25.6 | 26.6 | 25.3 | 25.4 | 25.9 | 25.0 | 25.1 | 25.4 | 27.1 | 26.2 | 26.4 | 25.5 | 24.1 | 26.0 | 25.1 | 27.2 | 26.2 | 27.4 | 26.9 | 27.9 | 27.2 |
| Temperature °C | 26.0 | 26.3 | 25.8 | 25.9 | 26.0 | 24.5 | 25.3 | 24.9 | 27.5 | 26.5 | 26.9 | 24.8 | 24.5 | 25.8 | 25.8 | 26.8 | 26.3 | 27.1 | 26.1 | 27.5 | 26.9 |
| Temperature °C | 26.1 | 27.0 | 25.0 | 24.8 | 26.1 | 24.0 | 24.6 | 24.8 | 26.9 | 26.9 | 25.6 | 25.0 | 24.8 | 26.1 | 26.0 | 26.5 | 26.1 | 26.9 | 26.4 | 26.8 | 27.1 |
| Distance of water (m) | 13.2 | 52.2 | 30.2 | 72.8 | 29.6 | 20.9 | 19.3 | 116.5 | 91.3 | 42.7 | 48.7 | 17.0 | 56.1 | 44.0 | 217.1 | 211.8 | 124.6 | 44.7 | 20.2 | 83.1 | 252.2 |
| Rainfall (mm) | 10.2 | 0 | 0.2 | 0 | 0 | 47.6 | 0 | 5.2 | 0.2 | 42.2 | 0 | 0 | 0.2 | 0 | 0.4 | 0 | 1.4 | 0.2 | 8.4 | 10.4 | 0.2 |

Suppl. Figure 1. Landscapes with their respective values of forest cover, wealth and abundance of mosquito species in Bodoquena Plateau, Mato Grosso do Sul, Brazil.



## Final Considerations

Identifying ecological factors and paths that affect the composition of species of epidemiological importance are essential because they inform vector integrated management strategies. We emphasize that larval control in habitats requires knowledge about mosquito ecology and the effects of biotic and abiotic variables on larvae, especially when it comes to biological controls. The application of integrated pest management can be conducted in both dry and rainy seasons. However, we recommend focusing on the dry season when larval habitats are more limited, in smaller volumes and more accessible for entry and application of vector control techniques. These are critically important considerations because over 2 million people live in Amazonas state (IBGE, 2020) and anophelines transmitted over 59,637 malaria cases in the Amazon region in the first half of 2020, and about 44.4% came from the state of Amazonas (MS, 2020).

In summary, we found strong evidence for the influence of forest cover on the abundance of mosquito species in the Cerrado. Information about mosquito distribution, particularly from this poorly studied region, is necessary to contribute to local and national vector surveillance strategies, including possible monitoring and risk assessment programs for the emergence of arboviruses and other diseases transmitted by mosquito vectors in Brazil.

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# Appendix

Academic production during the doctorate.

## Chapter 1 published

Arcos, A. N.; Ferreira, F. A. S.; Cunha, H. B.; Tadei, W. P. Characterization of artificial larval habitats of *Anopheles darlingi* (Diptera: Culicidae) in the Brazilian Central Amazon. *Revista Brasileira de Entomologia*, v. 62, p. 267-274, 2018. https://doi.org/10.1016/j.rbe.2018.07.006

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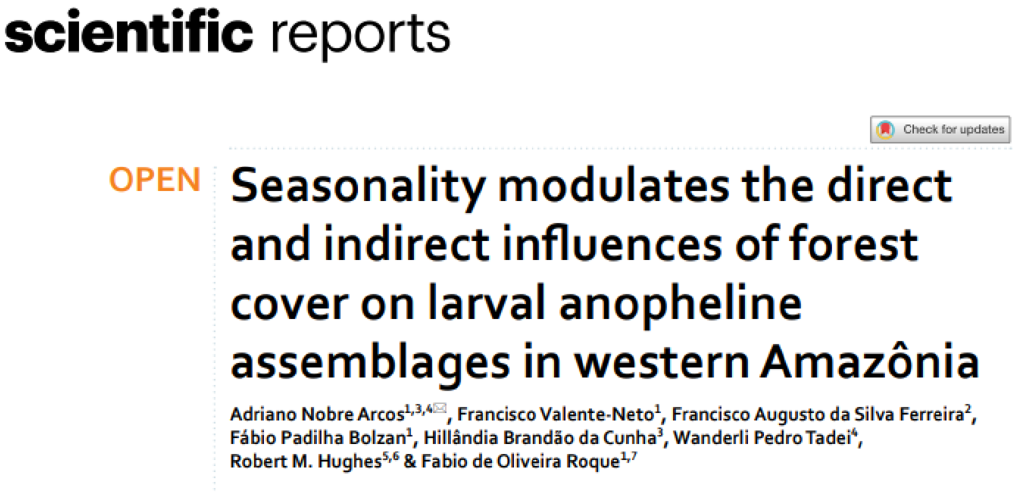
## Chapter 2 published

Arcos, A.N., Valente-Neto, F., da Silva Ferreira, F.A. et al. Seasonality modulates the direct and indirect influences of forest cover on larval anopheline assemblages in western Amazônia. Sci Rep 11, 12721 (2021). https://doi.org/10.1038/s41598-021-92217-9



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Seasonality modulates the direct and indirect influences of forest cover on larval anopheline assemblages in western Amazônia

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## Participation in academic and scientific events

Econexão - I Encontro do Programa de Pós Graduação em Ecologia e Conservação - UFMS. 2020.
Palestra: A importância dos Estudos de Potencial Malarígeno no Licenciamento Ambiental de grandes empreendimentos na Amazônia. 2020.
Simpósio Virtual de Limnologia e Saneamento do Semiário. 2020.
71ª Reunião Anual da SBPC. 2019.
Integra UFMS. 2019.
I Workshop Ecologia do Pantanal. 2019.
SAPECO - Semana Acadêmica do Programa de Pós Graduação em Ecologia e Conservação - UFMS. 2019.
Agroecol 2018 - 3 Seminário de Agroecologia de América do Sul. 2018.
XXVII Congresso Brasileiro e o X Congresso Latino-Americano de Entomologia. 2018.
SAPECO - Semana do Programa de Pós-graduação em Ecologia e Conservação - UFMS. 2017.
Symposium on Ecology and Evolution of Plant Reproduction. 2017.
V Plantando Educação e Colhendo Ciência - PECC. 2017.
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