

Ecological characterization of mosquitoes (Diptera: Culicidae) in areas of the Mato Grosso Pantanal, Mato Grosso State, Brazil

Jerônimo Alencar^{1*}, Vanessa Melandri, Júlia dos Santos Silva¹,
Hermano Gomes Albuquerque² and Anthony Érico Guimarães¹

¹Laboratório de Diptera, Instituto Oswaldo Cruz (Fiocruz), Manginhos, Rio de Janeiro, Brazil;

²Laboratório de Doenças Parasitárias, Instituto Oswaldo Cruz (Fiocruz), Manginhos, Rio de Janeiro, Brazil

Received for publication: 13 January 2021; Revision received: 26 March 2021;
Accepted for publication: 1 June 2021.

Abstract: Bimonthly diurnal and nocturnal captures were conducted in four different sampling sites of the Mato Grosso Pantanal region over 24 consecutive months between March 2009 and January 2011. The goal of performing these collections was to elucidate aspects of the biology and ecology of mosquitoes in this region since little to nothing is known about these aspects for many species. A total of 17,532 specimens were captured, comprising two subfamilies, nine genera, and 44 species. The main species of culicids found in the Mato Grosso Pantanal had strong ecological compatibility with well-preserved environments and with some of the specific habitats found in at least one of the four selected collection sampling sites. *Anopheles darlingi*, *An. albitarsis*, and *An. triannulatus* are well-suited to environments with large water bodies and greater anthropic activity. *Culex nigripalpus* and *Cx. quinquefasciatus* prefer environments with bush fragments and pronounced human action and movement. *Culex declarator* and *Psorophora albigena*, the most eclectic, prefer wild areas and with some anthropic activity. *Mansonia titillans* is associated with environments offering a wide variety of natural breeding areas that enable its development, in particular natural swamp areas covered with macrophytes, which is one of the natural components of the Pantanal biome. The effect of the flooding season on the population density of anophelines is especially advantageous, with an explosion in the number of individuals in periods of widespread flooding. The region's environmental dynamics are regulated by flooding cycles with alternating periods of flooding and drought, which are the main factors governing the ecology of the local fauna and flora. Flooding periods contribute to raising culicid species' richness and diversity, while the droughts result in a more even distribution of specimens within species. The flooding season of the Pantanal occurs between November and May and coincides with the greatest abundance of culicid species. During this period, the human population of the region has greater contact with mosquito vectors carrying pathogens, including arboviruses, which previous studies have confirmed via seropositivity in the region's equines and birds.

Key words: Culicidae; diversity; ecology; vectors; Pantanal biome.

*Corresponding author. E-mail: jalencar@ioc.fiocruz.br

©Copyright: the Author(s), 2021 | Licensee PAGEPress, Italy



Introduction

Mosquitoes play an important role in epidemiological risk studies due to their prevalence as hematophagous vectors. Their presence in urban areas has attracted greater interest and studies concerning their natural habitat. Culicidae species are an important vector for diseases, particularly in the case of arboviruses, a diverse family that includes dengue, yellow fever, Rocio, Ilheus, St. Louis, and West Nile viruses (WHO 2018). About 3,583 species of mosquitoes are currently recognized (<http://mosquito-taxonomic-inventory.info/simpletaxonomy/term/6045>), distributed among approximately 113 genera (Harbach 2020). The Neotropical region has the highest endemicity level, with 27% of these species restricted to this biogeographic region (Ward 1984).

The Mato Grosso Pantanal is the planet's largest continuous wetland, spanning approximately 140,000 km². This area is a UNESCO World Biosphere Reserve and is part of the hydrographic sub-basin of the Paraguay River. Located in South America, it includes territory in Brazil, Bolivia, and Paraguay. Roughly 85% of the Pantanal occurs in Brazil, with 35% in the Mato Grosso (MT) state, referred to as the northern Pantanal, and 65% in the Mato Grosso do Sul (MS) state, known as the southern Pantanal (Alho *et al.* 1988). The northern and southern Pantanal are divided into several sub-regions. This study was conducted in the Poconé sub-region of the Pantanal, which covers approximately 11% of the Brazilian Pantanal (Nunes and Tomas 2008). The area is characterized by seasonal habitat change with a wet, flooding period between December and May and a dry season between June and November. This climate pattern directly influences the region's ecology, controlling both biotic and abiotic processes on the plain (Nunes da Cunha and Junk 2004).

The Pantanal has several characteristics that facilitate and maintain the presence of arboviruses. This includes the area's rich wildlife diversity, such as resident and migratory birds, and the regional climate factors that favor the proliferation of mosquito-borne diseases (Ward 2005). These characteristics make the region a key area for entomological monitoring and one of the main areas for studying enzootic cycles in Brazil.

This study aims to inventory and evaluate the abundance of the culicid fauna during two well-defined climatic seasons (rainy summer and dry winter) in four previously selected areas of the Mato Grosso Pantanal.

Materials and methods

The region's complex relief consists of typified formations, including (a) baías or bays, which can be circular and often contain brackish water with up to 100 m between banks; (b) cordilheiras or ridges of about 2 m in height, generally located between baías, which are usually home to the farms and cattle shelters; (c) vazantes or lagoons, sheltered areas of bays and rivers caused by receding floodwaters, which may be transient or perennial and can reach several kilometers in extent; and (d) corixos or channels, short courses of water that interlink the baías and result in high erosive action (IBGE, 1988). Though there are climatic differences between different sub-regions, the regional climate has two distinct seasons: the rainy season, which runs from late November to early May, and the dry season. The alternation of lengthy droughts and major flooding is the regulating mechanism that exerts complete control over the activity of most living beings that inhabit the Pantanal (Moraes *et al.* 2000).

The studied areas are located along the Parque Transpantaneira Route (EPT) between the localities of Poconé in the Mato Grosso state and Porto Jofre, on the border of the Mato Grosso do Sul state, in the area known as Pantanal de Poconé (Pantanal do Cuiabá – Bento Gomes – Paraguaizinho). In this study, four sampling sites identified as A, B, C, and D (Figure 1) were selected in the same region of the Mato Grosso Pantanal, comprising the

areas most affected by the region's cycles of inundation, flooding, receding, and drought. During the receding phase, floodwater flows down through the natural riverbeds. The sampling sites were as follows:

Capture sampling site A – Coordinates: 16°39'54.3" S; 56°47'38.2" W. Located at the side of the EPT, the area experiences intense flooding during the rainy season and a proportionally arid phase during the dry season. The vegetation community is similar to a steppe-like savannah, with small thorny bushes and trees that are resistant to the dry soil and offer shelter for local fauna.

Capture sampling site B – Coordinates: 16°44'56.3" S; 56°51'32.6" W. Located where the EPT meets the Pixaim River, this sampling site is directly influenced by the anthropic activity of the Hotel Pantanal de Mato Grosso and is an area less affected by flooding.

Capture sampling site C – Coordinates: 16°51'10.2" S; 56°50'06.8" W. Located at the side of the EPT, its vegetation is typical of the Cerrado biome, with medium-sized shrubs. The entire area is densely covered by a vast carpet of grasses (tabocas), which are completely flattened during the floods.

Capture sampling site D – Coordinates: 16°53'30.1" S; 56°50'37.3" W. Located at the side of the EPT, its vegetation is similar to a gallery or riparian forest. This site has a wealth of mosquito breeding sites in the hollows of the largest trees, even during the long drought periods. This is the most representative area of the Pantanal seasonal deciduous forest, with dense vegetation interlinked by the treetops and an abundance of bromeliads and other epiphytes.

Sampling was carried out every two months between March 2009 and January 2011 for 24 consecutive hours comprising a day and night period. In each sampling period, mosquitoes were captured over five days using CDC-type light traps. The traps were kept active between 6 pm and 6 am of the following day, with attraction by an incandescent or LED light source. During the day, mosquitoes were attracted using a lure indicating human presence and CO₂, which was activated at dawn and dusk. All captured mosquitoes were killed by exposure to low temperatures and immediately packed in polyethylene containers grouped according to the conditions under which they were sampled. The containers were sequentially numbered based on field conditions and taken to the lab for screening and identification of mosquitoes. Specimens were cataloged in the Entomological Collection of the Oswaldo Cruz Institute, under the title "Mato Grosso Pantanal collection".

Species identification was made by directly observing their morphology with a stereoscopic microscope (ZEISS Steme SV6) and consulting dichotomous keys developed by (Forattini 2002; Consoli and Lourenço-de-Oliveira 1994; Lane 1953). The abbreviations of genus and subgenus names are consistent with those described by Reinert (2001).

The data were processed in order to express the ecological relationship between mosquito populations and the environmental factors of the study area. To assess diversity and whether the species had a homogeneous distribution, the Shannon-Weaner diversity (H') and equitability (EH) indices were used with all captured mosquitoes, including those representing less than 5% of the total. The influence of climatic factors on the culicid fauna was analyzed using linear correlation coefficients with a 95% confidence limit for $r=0.51$.

The phases of the annual climate regime of the Pantanal region are of great importance since they determine biotic and abiotic conditions that influence the biogeographic conditions of the study region. The comparison between the study area's rainy and dry seasons was carried out using the diversity indices, the standardized index of species abundance (ISA-SISA), as well as estimations of similarity using a t-test. A Student's t-test was used to compare independent groups to assess the degree of similarity between culicid species present at different capture sampling sites. Differences between species within the groups

were considered significant for $p < 0.05$ within a 95% confidence interval. We used ArcGIS 10 software (Environmental Systems Research Institute, Redlands, CA) to perform the spatial analyses.

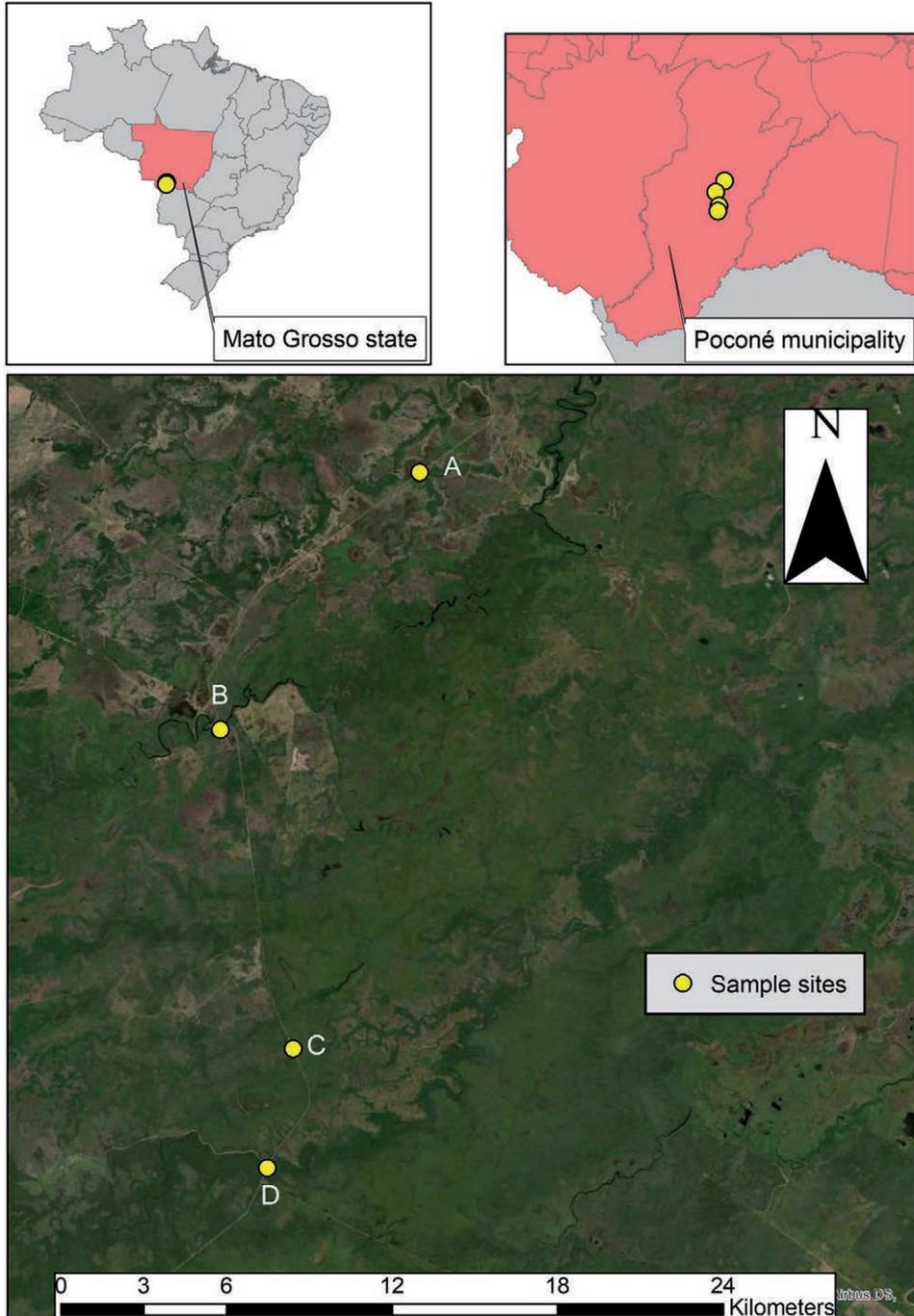


Figure 1. Location of the study area and sampling sites in the Mato Grosso Pantanal, Mato Grosso state, Brazil.

Results

A total of 17,532 specimens were captured, comprising two subfamilies, nine genera, and 44 species. The subfamily Anophelinae was represented by the genus *Anopheles* Meigen, 1818 with 11 species, and the subfamily Culicinae by eight genera and 33 species (Table 1). About 78% of all mosquito specimens belonged to the subfamily Culicinae, of which *Culex* Linnaeus, 1758 accounted for 60% of the total sample, while 22% belonged to the subfamily Anophelinae.

The daytime CDC traps, which used a human presence simulator lure and CO₂, captured practically no specimens of the entirely diurnal mosquitoes *Sabethes* Robineau-Desvoidy, 1827. The following species were limited to rare occurrences and therefore not considered in subsequent analyses: *Sabethes albiprivus* Theobald, 1903, *Sa. purpureus* Theobald, 1907, and *Sa. chloropterus* Humboldt, 1819.

The subfamily Anophelinae was mainly represented by *Anopheles evansae* (26.5%), followed by *An. triannulatus* (20.8%), *An. albitarsis* (18.6%), *An. matogrossensis matogrossensis* (17.1%), *An. rondoni* (8.0%), and *An. darlingi* (7.6%). Over 70% of the Culicinae specimens present are from the genus *Culex*. This subfamily was represented mainly by *Culex nigripalpus* (24.7%), followed by *Cx. declarator* (23.6%), *Psorophora albigenu* (15.8%), *Cx. quinquefasciatus* (11.6%), and *Mansonia titillans* (8.9%) (Table 1).

The most favorable months for mosquitoes were March 2009 (2,873 specimens) and January 2011 (4,927 specimens), in which their abundance was outside the expected range. Meanwhile, the lowest population density was recorded in November 2009 (198 specimens) and November 2010 (255 specimens) (Table 1). All species of Anophelinae showed a distribution pattern in terms of their abundance over time. The May 2009 sampling was the most abundant, with peaks above the tolerance range for *An. albitarsis*, *An. darlingi*, *An. matogrossensis*, *An. rondoni*, and *An. triannulatus* (Figure 2). The highest abundance of *An. evansae* was in March 2010, also above the tolerance range. These species also exhibited a uniform pattern of absences, with *An. albitarsis*, *An. darlingi*, *An. evansae*, *An. matogrossensis*, *An. rondoni*, and *An. triannulatus* absent in several collections between September and January in both 2009 and 2010 (Figure 2).

The subfamily Culicinae had their highest abundance in January 2011, with peaks above the tolerance range for *Cx. declarator*, *Cx. nigripalpus* and *Cx. quinquefasciatus*. The highest abundance of *Ma. titillans* was in May 2009 and that of *Ps. albigenu* in January 2010. On the other hand, the subfamily was less frequent in other sampling months. *Culex declarator* and *Cx. nigripalpus* were less common in November 2009 and July 2010, *Cx. quinquefasciatus* was absent in March 2009 and November 2010, *Ma. titillans* was under-represented in November 2009 and January 2011, and *Ps. albigenu* was not present in September 2010 or November 2010 (Figure 2). The most abundant species in the study area belong to the Culicinae, followed by the Anophelinae. *Culex nigripalpus* (SISA=0.73) was the most abundant, followed by *Cx. declarator* (SISA=0.69) and *Ma. titillans* (SISA=0.65).

The species distributions also changed at each of the capture sampling sites. At capture sampling site A, the most abundant species was *Cx. nigripalpus* (SISA=0.72), followed by *Ps. albigenu* (SISA=0.58) and *Cx. declarator* (SISA=0.50).

At capture sampling site B, *Cx. nigripalpus* (SISA=0.89) was the most abundant species, followed by *An. albitarsis* (SISA=0.54) and *Cx. declarator* (SISA=0.50). At capture sampling site C, *Cx. declarator* (SISA=0.83) was the most abundant, followed by *Cx. quinquefasciatus* (SISA=0.54) and *Ps. albigenu* (SISA=0.53). Finally, at capture sampling site D, the most abundant species was *Cx. nigripalpus* (SISA=0.73), followed by *Cx. declarator* (SISA=0.58) and *An. evansae* (SISA=0.53).

Table 1. Seasonal distribution of mosquitoes found in areas of the Mato Grosso Pantanal in the state of Mato Grosso, Brazil, in absolute values (N) and percentages (%) by species throughout the sampling efforts between March 2009 and January 2011.

Species	2009			2010			2011			Total						
	Mar	May	Jul	Sep	Nov	Jan	Mar	May	Jul	Sep	Nov	Jan	N	%	%	Subfamily Total
Subfamily Anophelinae																
<i>Anopheles albitarsis</i> Lynch Arribalzaga, 1878	208	306	18	0	0	5	112	27	30	1	0	13	720	18.6	4.1	
<i>Anopheles darlingi</i> Root, 1926	25	130	5	1	0	1	32	34	45	0	0	22	295	7.6	1.7	
<i>Anopheles evansae</i> (Brèthes, 1926)	2	120	72	2	0	16	767	5	31	1	0	9	1025	26.5	5.8	
<i>Anopheles galvaoi</i> Causey, Deane & Deane, 1943	0	2	3	2	0	0	0	0	0	0	0	0	7	0.2	0.0	
<i>Anopheles matogrossensis</i> Lutz & Neiva, 1911	3	359	131	8	1	10	30	73	45	0	0	0	660	17.1	3.8	
<i>Anopheles oswaldoi</i> (Peryassú, 1922)	12	21	0	0	1	0	1	1	0	0	0	0	36	0.9	0.2	
<i>Anopheles rangeli</i> Gabaldon, Cova García & Lopez, 1940	1	1	1	0	0	0	0	0	0	0	0	0	3	0.1	0.0	
<i>Anopheles rondoni</i> (Neiva & Pinto, 1922)	7	187	1	0	0	0	27	20	64	3	0	0	309	8.0	1.8	
<i>Anopheles strodei</i> Root, 1926	0	4	2	0	0	0	0	0	0	0	0	0	6	0.2	0.0	
<i>Anopheles triannulatus</i> (Neiva & Pinto, 1922)	161	201	37	8	7	0	60	107	195	23	0	4	803	20.8	4.6	
Subtotal	419	1331	270	21	9	32	1029	267	410	28	0	48	3865	100.0	22.0	
Subfamily Culicinae																
<i>Aedeomyia squamipennis</i> (Lynch Arribalzaga, 1878)	23	2	1	0	3	74	2	6	0	3	0	13	127	0.9	0.7	
<i>Coquillettidia chrysonotum</i> (Peryassú, 1922)	0	0	0	0	6	0	0	2	0	0	0	0	8	0.1	0.0	
<i>Coquillettidia fasciolata</i> Lynch Arribalzaga, 1891	1	0	2	0	0	0	0	2	0	2	0	10	17	0.1	0.1	
<i>Coquillettidia juxtamansonia</i> (Chagas, 1907)	1	6	0	0	0	0	0	0	0	7	0	93	107	0.8	0.6	
<i>Coquillettidia shannoni</i> Lane & Antunes, 1937	52	85	5	21	60	169	43	6	0	0	0	0	441	3.2	2.5	
<i>Coquillettidia venezuelensis</i> (Theobald, 1912)	0	0	1	0	0	0	0	0	62	2	0	0	65	0.5	0.4	
<i>Culex declarator</i> (Dyar & Knab, 1906)	723	277	93	215	12	102	325	53	21	23	43	1335	3222	23.6	18.4	
<i>Culex nigripalpus</i> Theobald, 1901	801	316	106	246	14	98	103	61	24	26	60	1526	3381	24.7	19.3	
<i>Culex quinquefasciatus</i> Say, 1923	0	38	66	154	9	20	110	198	15	16	0	954	1580	11.6	9.0	

To be continued on next page

Table1. Continued from previous page.

Species	2009			2010			2011			Total Subfamily Total						
	Mar	May	Jul	Sep	Nov	Jan	Mar	May	Jul		Sep	Nov	Jan	N	%	
Subfamily Culicinae																
<i>Mansonia humeralis</i> (Dyar & Knab, 1916)	40	48	6	1	0	0	0	6	0	0	16	0	5	122	0.9	0.7
<i>Mansonia indubitans</i> (Dyar & Shannon, 1925)	4	32	25	0	0	17	1	0	0	0	0	0	16	95	0.7	0.5
<i>Mansonia titillans</i> (Walker, 1848)	227	264	189	78	44	118	47	74	92	58	5	17	1213	8.9	6.9	6.9
<i>Ochlerotatus fluviatilis</i> (Lutz, 1904)	0	0	0	0	0	0	0	0	15	0	0	0	0	15	0.1	0.1
<i>Ochlerotatus fulvithorax</i> (Lutz, 1904)	2	0	0	0	0	0	0	0	0	0	0	0	0	2	0.0	0.0
<i>Ochlerotatus fulvus</i> (Wiedmann, 1828)	0	1	0	0	0	3	3	0	0	0	0	0	0	7	0.1	0.0
<i>Ochlerotatus ryacophilus</i> Costa Lima, 1933	0	0	9	0	0	0	0	0	0	0	0	0	0	9	0.1	0.1
<i>Ochlerotatus scapularis</i> (Rondani, 1848)	6	0	1	4	26	0	2	1	45	3	50	410	548	4.0	3.1	3.1
<i>Ochlerotatus serratus</i> (Theobald, 1901)	5	1	0	0	1	8	1	0	0	0	0	0	0	16	0.1	0.1
<i>Psorophora albigena</i> (Peryassú, 1908)	497	32	248	84	9	779	26	10	15	0	0	0	458	2158	15.8	12.3
<i>Psorophora ciliata</i> (Fabricius, 1794)	3	0	0	0	0	0	1	0	0	0	0	0	36	40	0.3	0.2
<i>Psorophora discrucians</i> (Walker, 1856)	3	0	0	17	0	0	0	1	0	0	93	0	114	0.8	0.7	0.7
<i>Psorophora ferox</i> (von Humboldt, 1820)	0	2	0	0	5	1	0	0	0	0	1	6	15	0.1	0.1	0.1
<i>Uranotaenia apicalis</i> Theobald, 1903	0	0	0	0	0	0	0	0	0	2	0	0	2	0.0	0.0	0.0
<i>Uranotaenia calosomata</i> (Dyar & Knab, 1907)	0	0	0	0	0	0	0	0	0	4	0	0	4	0.0	0.0	0.0
<i>Uranotaenia ditaenionota</i> Prado, 1931	24	1	0	0	0	0	0	0	0	0	0	0	25	0.2	0.1	0.1
<i>Uranotaenia geometrica</i> (Theobald, 1901)	0	7	0	0	0	0	1	0	0	0	0	0	8	0.1	0.0	0.0
<i>Uranotaenia leucoptera</i> (Theobald, 1907)	0	0	1	0	0	0	0	0	0	0	0	0	1	0.0	0.0	0.0
<i>Uranotaenia lowi</i> Theobald, 1901	0	2	0	0	0	2	1	0	0	0	3	0	8	0.1	0.0	0.0
<i>Uranotaenia pallidiventer</i> Theobald, 1903	3	0	6	0	0	0	0	0	0	0	0	0	9	0.1	0.1	0.1
<i>Uranotaenia pulcherrima</i> (Lynch Arribálzaga, 1891)	38	269	1	1	0	0	0	0	0	0	0	0	309	2.3	1.8	1.8
Subtotal	2452	1383	760	821	189	1391	672	414	289	162	255	4879	13668	100.0	78.0	78.0
Total	2872	2714	1030	842	198	1423	1701	681	699	190	255	4927	17532		100.0	

An analysis of the distribution of each genus over the landscape showed that samples of *Coquillettidia* Dyar, 1905 were predominated by *Coquillettidia shannoni*, except for sample area D, where *Cq. juxtamansonia* was also found in abundance.

The *Culex* species appear to be evenly distributed across the sample areas, with the relative proportion of each species roughly similar at each site. *Culex declarator* and *Cx. nigripalpus* were the most abundant in the sample areas, with *Cx. quinquefasciatus* always found in smaller proportions. The distribution of the genus *Mansonia* Blanchard, 1901 was fairly homogeneous throughout the sample areas. *Mansonia titillans* was the predominant species of the genus in all sample areas, with *Ma. humeralis* and *Ma. indubitans* present in lower numbers.

The distribution of the genus *Ochlerotatus* Lynch Arribálzaga, 1891 was heterogeneous, though *Oc. scapularis* constantly predominated. This is the only species of the genus present in the sample area C, while other species of *Ochlerotatus* were abundant in differing proportions at the other sites.

The distribution patterns of *Psorophora* Robineau-Desvoidy, 1827 and *Uranotaenia* Lynch Arribálzaga, 1891 were similar to those of *Ochlerotatus*, with heterogeneity in the species distribution among the sampling areas. However, *Ps. albigena* and *Ur. pulcherrima* predominated relative to their respective congeneric species.

Anophelines had the greatest heterogeneity in species distribution throughout the four sample areas. The sampling sites A and B were the ones with a similar species distribution, with sites C and D differing from one another and from sites A and B (Figure 2).

Only *Ps. albigena* had a statistically significant difference in its abundance between sampling sites A and B ($p=0.046$), with no difference detected between all other species at these sampling sites. In addition, no significant differences in species abundance were found between sites A and C, given a threshold of $p=0.05$ within a 95% confidence interval. However, there were significant differences in the abundance of *An. albitarsis* ($p=0.048$), *Cx. declarator* ($p=0.023$), and *Ps. albigena* ($p=0.016$) between sites A and D. The only species whose abundance differed between sampling sites B and C was *Ps. albigena* ($p=0.016$). Only the abundance of *An. darlingi* differed significantly between sampling sites B and D ($p=0.035$). Finally, *Cx. declarator* ($p=0.029$) and *Ps. albigena* ($p=0.006$) had significant differences in their abundance between sampling sites C and D.

There was no significant difference in species richness and diversity according to the Shannon-Weaner diversity index. The sampling site A had the highest number of specimens collected ($n=4,897$) and the lowest equitability ($EH=0.31$). Sites B and C had the highest richness ($S=33$), while site D had the lowest richness ($S=26$), the lowest number of captured specimens ($n=3,058$), and the highest equitability index (0.42), indicating a more even distribution of species abundance at the site.

Using the SISA abundance index between the rainy and dry seasons, changes in the species' rankings were observed between the periods of drought and rains. The most abundant species in the dry season was *Cx. nigripalpus* (SISA=0.74), followed by *Ma. titillans* (SISA=0.73), *Cx. declarator* (SISA=0.65), and *An. triannulatus* (SISA=0.38). In the rainy season, the most abundant species was *Cx. declarator* (SISA=0.64), followed by *Cx. nigripalpus* (SISA=0.60), *Ma. titillans* (SISA=0.50), and *Ps. albigena* (SISA=0.48).

Given the significant direct and indirect effects of the seasonal regime of alternating rains and droughts throughout the Pantaneira Plains, it is noteworthy that the frequency of all species was greater than 60% in the rainy season, with some reaching a frequency of around 90% in the flooding period, as in the case of *An. albitarsis*.

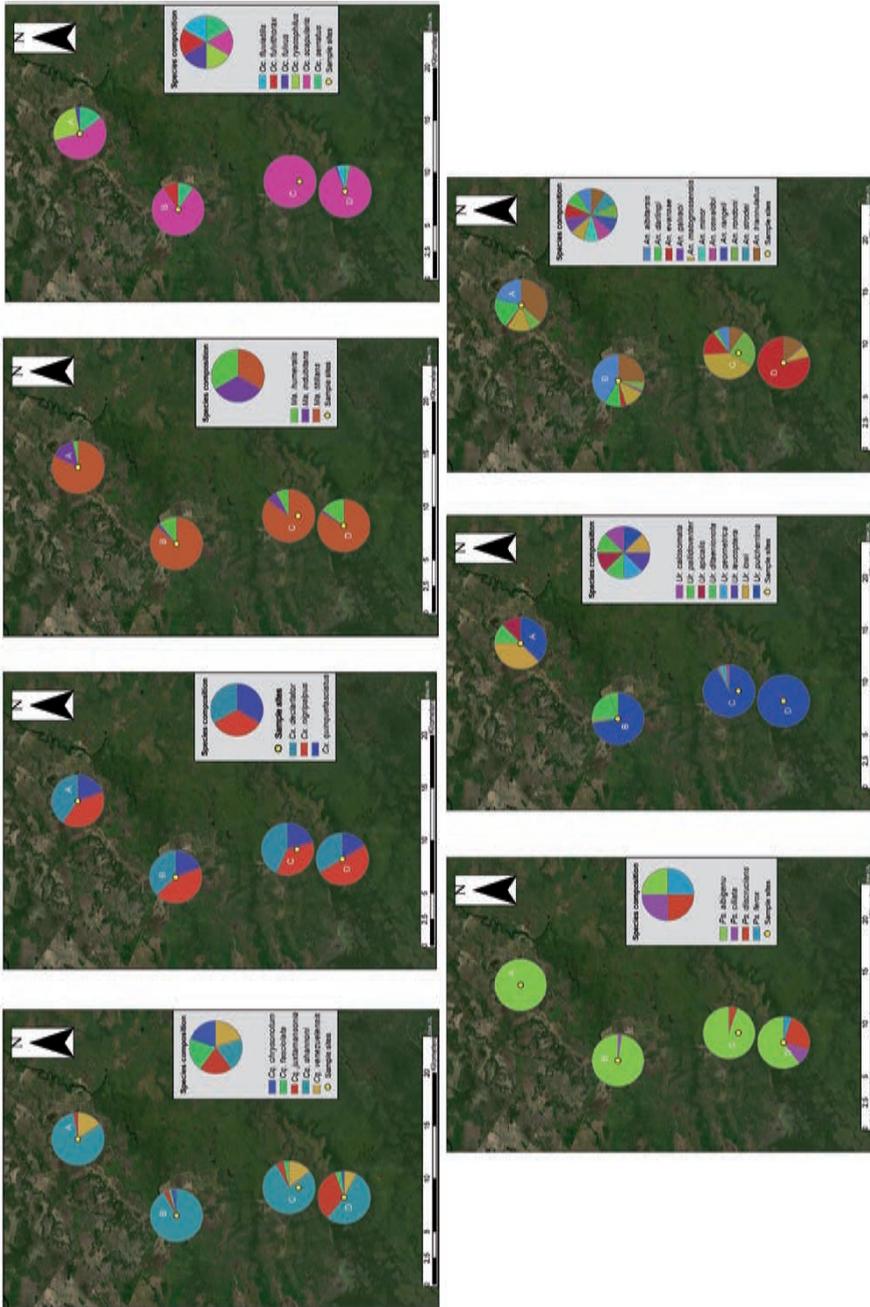


Figure 2. The ten most representative species at each sample site, with the greatest effect on the mosquito community composition using the ArcGIS 10 software (Environmental Systems Research Institute, Redlands, CA) for the spatial analyses.

Differences between species within the groups were considered significant according to the Student's t-test for $p < 0.05$ within a 95% confidence interval. The difference in frequency between the rainy and dry seasons was only significant for *An. albitarsis* ($p = 0.033$) and *Cx. declarator* ($p = 0.037$). Therefore, there was no difference in the frequency of the other species between the rainy and dry seasons according to the comparison of the means.

When analyzing the samples collected during each rainy season, we observed differences between the collections. The highest value of the Shannon–Weaner diversity index was observed in May 2009 ($H = 2.57$), a period of transition between the rainy and dry seasons, at which time we also observed the highest richness ($S = 27$) and the third-highest abundance ($n = 2,714$) of any collection period.

The lowest equitability index was observed during the rainy season in March 2009 ($EH = 0.27$), when the second-largest number of specimens were collected ($n = 2,873$). Meanwhile, the lowest diversity index was found during the dry season in November 2010 ($H = 1.48$), with the lowest richness ($S = 7$) and the second-lowest number of collected specimens ($n = 255$). These findings reveal that the rainy season had a higher species richness and number of individuals captured but lower diversity and equitability indices than the dry season.

Discussion

The Mato Grosso Pantanal is situated on a large plain with an environmental dynamic regulated primarily by heavy rains that drive intense flooding and drought periods. This climatic regime constitutes the principal factor regulating the ecology of the local flora and fauna (Barros 2001; Martins *et al.* 2004; Marques *et al.* 2006). Variations in annual rainfall greatly affect the biota, with different intensity and time scales (Ravazzani *et al.* 1990).

These cycles alternate between flooding periods, which offer the greatest opportunity for breeding, and the dry season, where breeding occurs in small, local habitats that persist after the wetlands drain or in areas with strong human influence. The alternation between flooding and dry periods leads rainfall to be a decisive factor for the majority of the region's mosquitoes (Barros 2001; Martins *et al.* 2004; Marques *et al.* 2006). Humans in this forested area come into greater contact with arbovirus-carrying mosquitoes during the flooding season, when culicid species occur more frequently (Iverson *et al.* 1993).

In contrast to Brazilian biomes such as the Cerrado and the Atlantic and Amazon forests, the Pantanal fauna is not characterized by the presence of many endemic species (Britski *et al.* 1999). However, the specific characteristics of the vegetation and the annual rainfall regime cause long periods of drought and flooding. These cycles propitiate plentiful natural refuges and food, making the Pantanal an important area for the reproduction of many migratory species, especially birds (Cordivola de Yuan 1992; Junk *et al.* 1989; Mauro 1993). Several of these birds are native to other countries, and some carry mosquito-borne pathogens. Thus, the study of the occurrence and ecology of these vectors is fundamental. For instance, Melandri *et al.* (2012) reported the West Nile virus in birds and horses living in farms near the sample sites analyzed in this study.

The abundance of the subfamily Anophelinae peaked in May 2009 and 2010, while also rising above the upper tolerance range in other periods. The period from April to May marks the end of the impact of major rains in the region and can be considered a transitional stage between the rainy and dry seasons, with extensive areas remaining under maximum flooding risk for some time. This phase is very favorable for species that use floodwaters for oviposition and rearing their immature forms (Berti *et al.* 1993; Natal *et al.* 1995).

In addition to the abundance pattern in May, *Anopheles triannulatus* also showed a marked peak in July, which represents the height of the dry season in the region. We find similar trends in reports by Deane et al. (1948), who noted that some anopheline species can have population peaks in the season because the streams are reduced to smaller and slower creeks or isolated pools where their larvae grow well.

The subfamily Anophelinae had a similar distribution pattern. We detected a uniform behavior of anophelines, with peaks above the tolerance range during flooding months due to high rainfall rates. This finding is consistent with the study by Quintero et al. (1996), who observed an increase in the population of anophelines during flooding periods after the formation of the reservoir of the Balbina Hydroelectric Plant in the Amazonas state, Brazil. It is important to highlight the great heterogeneity of the Anophelinae among the collection sampling sites, a finding similar to that of Martins et al. (2018). The species may be sensitive to different landscapes, resulting in contrasting species compositions according to the habitat and landscape.

Among the culicid species found in the four study areas in the Mato Grosso Pantanal, some have been reported as vectors of human and/or animal pathogens. Of these, we highlight the high abundance of *Cx. quinquefasciatus* and *Cx. nigripalpus*, which are important vectors of the West Nile virus in North America, especially in urban and rural areas of the United States.

Species of the genus *Culex* had some similarities in their monthly population density trends, with peaks above the tolerance range in March 2009 in *Cx. declarator* and *Cx. nigripalpus*, and a marked increase in January 2011 for the two previous species and *Cx. quinquefasciatus*. In studies on mosquito feeding preferences in the same region, Alencar et al. (2005) observed that female mosquitoes tested with precipitin had a greater response for birds as a food source, especially *Cx. nigripalpus* and *Ma. titillans*.

Guimarães and Arlé (1984), Guimarães et al. (2000), and Forattini et al. (1995) reported the same distribution trend found in this study for *Cx. nigripalpus* and *Cx. declarator*, with a population decrease in the coldest and driest months and a peak in the period of strong rainfall.

Mansonia titillans had a population density above the tolerance range in March 2009 and May 2009, decreasing in November 2010. Its presence is directly associated with aquatic vegetation since the species occurs in aquatic vegetation during its immature stages following the first larval stage to withdraw oxygen for breathing directly from the aeriferous parenchyma (Consoli and Lourenço-de-Oliveira 1994 and Forattini 2002). Thus, the population peaks above the tolerance range in March 2009 and May 2009 are likely due to more plentiful areas with aquatic vegetation due to the recent rainfall, resulting in more abundant breeding areas for this species.

The abundance of *An. darlingi* in sampling sites A and B is of particular interest, given the increasing rates of human settlement in the region. In addition, this species is the main transmitter of human malaria in Brazil, with high infection rates and significant capacity for transmission in the wild and built areas, even at low densities. Such abundance may be due to the fact that *An. darlingi* is the anopheline that best and most quickly benefits from anthropic changes, including agricultural and livestock exploration or even ecotourism, as observed in recent decades in the Pantanal (Vittor et al. 2006). Deane (1989), Tadei and Costa (1985) and Oliveira-Ferreira et al. (1990) cite alterations in the behavior of *An. darlingi* in Amazon regions with recent colonization, where mosquitoes began to demonstrate a major tendency to bite humans indiscriminately inside and outside their homes.

Anopheles albitarsis, the most abundant anopheline at sampling site B, is considered to be the main secondary vector of the pathogens that cause malaria throughout Brazil, in-

cluding the southeastern region, where it has already largely been transmitted by *An. darlingi* (Deane 1986; Teodoro *et al.* 1995; Guimarães *et al.* 1997 and Tadei and Thatcher 2000). More recently, *An. albitarsis* has been identified as the main vector of malaria in certain localities, including areas of the Serra do Navio in the Amapá state (Póvoa *et al.* 2001) and the region of Boa Vista, in the Roraima state (Silva-Vasconcelos *et al.* 2002).

Culex nigripalpus favored sampling sites A and B, which are places surrounded by an environment with bush fragments. In addition, sites A and B largely comprise areas affected by human activity and are covered with large grasses, which can be intensely flooded. The abundance of *Cx. nigripalpus* at these sites may also be related to their high tendency to feed on birds. They may be attracted by domestic birds at sampling site A and by the abundance of resident and/or migratory birds in the extensive flooded areas of sampling site B, especially during reproduction periods. This preference for biting birds and their relative distaste for human blood have been reported by Guimarães and Arlé (1984) and Forattini *et al.* (1993), who noted that many specimens remained in the surrounding vegetation for a long time before attacking the human lure. The occurrences of *Cx. declarator* and *Ps. albigena* found at sites in areas with some human activity (sampling sites A and B) and in exclusively wild areas (sampling sites C and D) demonstrate a likely eclectic behavior of the species with respect to host selection for hematophagy, with the choice of area influenced by the availability of places for egg-laying, which can change throughout the year. Forattini (2002) noted that both species have a certain tendency to adapt to the human environment.

The flooded areas covered by aquatic plants seem to have favored the proliferation of species of the Mansoniini tribe, which are generally very numerous and aggressive (Consoli and Lourenço-de-Oliveira 1994 and Forattini 2002). In this study, the significant presence of *Ma. titillans* in sampling sites A, B, and C is related to the availability of natural breeding areas with suitable vegetation, a natural component of the Pantanal. It should be noted that the distribution of the species of this tribe was quite homogeneous throughout the sample areas, indicating that the species may have a low sensitivity to habitat changes. This homogeneity pattern in the distribution of the species was also found for the genera *Psorophora*, *Uranotaenia*, and *Ochlerotatus*, in which the abundance of one species is generally accompanied by low densities of the other species.

Melandri *et al.* (2015) observed a 37-fold increase in the total occurrence of anopheline species influenced by large floods during the formation of the Serra da Mesa Hydroelectric Power Plant's artificial reservoir compared to the period before flooding. These results are consistent with those found in this study for the Matogrosso Pantanal, in that the anopheline species analyzed occurred mainly during the rainy season, particularly *An. albitarsis* (93%), *An. evansae* (90%), and *An. darlingi* (83%).

In the present study, we confirmed that the species diversity of the mosquito fauna is influenced by the local climatic factors of the biome, especially by the annual rainfall regime, with the highest abundance of mosquitoes occurring during the flooding season in the Pantanal. The human population currently inhabiting the region is subject to greater contact with culicid vectors that can carry pathogenic agents causing illnesses like arboviruses.

Contributions

AEG, JA, and JSS produced the idea, collected the data, contributed to the drafting and critical review of the content; HGA analyzed the spatial distribution of mosquitoes. All authors revised and approved the various drafts.

Conflict of interest

None

Funding

We thank the Furnas Centrais Elétricas for funding support.

References

- Alencar J, Lorosa ES, Silva JS, Lopes CM, Guimarães AE. 2005. Observations on feeding patterns among mosquitoes (Diptera: Culicidae) in the Pantanal, MT, Brazil. *Neotropica Entomology*. 34:681-687. <https://doi.org/10.1590/S1519-566X2005000400020>
- Alho CJR, Lacher TE, Gonçalves HC 1988. Environmental Degradation in the Pantanal Ecosystem. *BioScience*. 38:164-171. <https://doi.org/10.2307/1310449>
- Barros ATM. 2001. Seasonality and relative abundance of Tabanidae (Diptera) captured on horses in the Pantanal, Brazil. *Memórias do Instituto Oswaldo Cruz*. 96:917-923. <https://doi.org/10.1590/S0074-02762001000700006>
- Berti J, Zimmerman R, Amarista J. 1993. Spatial and temporal distribution of anophelinae larvae in two malarious áreas in Sucre State, Venezuela. *Memórias do Instituto Oswaldo Cruz*. 88:353-62. <https://doi.org/10.1590/S0074-02761993000300003>
- Britski HA, Silimon KZS, Lopes BS. 1999. Peixes do Pantanal. Manual de identificação. Brasília: Embrapa-SPI, Corumbá: Embrapa-CPAP. 184 pp.
- Consoli RAGB, Lourenço-de-Oliveira R. 1994. Principais mosquitos de importância sanitária no Brasil. Rio de Janeiro: Editora Fiocruz. 228 pp.
- Cordiviola de Yuan E. 1992. Fish populations of lentic environments of the Parana River. *Hydrobiologia*. 237:159-173. <https://doi.org/10.1007/BF00005848>
- Deane LM. 1986. Malaria factors in Brazil. *Memórias do Instituto Oswaldo Cruz*. 81:5-14. <https://doi.org/10.1590/S0074-02761986000600002>
- Deane LM, Causey OR, Deane MP. 1948. Notas sobre a distribuição e a biologia dos anofelinos das regiões nordestina e amazônica do Brasil. *Revista do Serviço Especial de Saúde Pública*. 1:827-965.
- Deane LM 1989. A cronologia da descoberta dos transmissores da malária na Amazônia Brasileira. *Memórias do Instituto Oswaldo Cruz*. 84:149-156. <https://doi.org/10.1590/S0074-02761989000800030>
- Forattini OP. 2002. Culicidologia médica: identificação, biologia, epidemiologia. Vol. II. São Paulo: Editora da Universidade de São Paulo, 860 pp.
- Forattini OP, Kakitani L, Massad E, Gomes AC. 1993. Studies on mosquitos (Diptera: Culicidae) and anthropic and environment. 1- Parity of blood seeking *Anopheles (Kerteszia)* in South-Eastern Brazil. *Revista de Saúde Pública*. 27:1-8. <https://doi.org/10.1590/S0034-89101993000100001>
- Forattini OP, Karitani L, Massad E, Marucci D. 1995. Studies on mosquitoes (Diptera: Culicidae) and anthropic environment. 10- Survey of adult behaviour of *Culex nigripalpus* and other species of *Culex (Culex)* in SouthEastern Brazil. *Revista de Saúde Pública*. 29:271-278. <https://doi.org/10.1590/S0034-89101995000400003>
- Guimarães AE, Arlé M. 1984. Mosquitos no Parque Nacional da Serra dos Órgãos, Estado do Rio de Janeiro, Brasil. I- Distribuição Estacional. *Memórias do Instituto Oswaldo Cruz*. 79:309-323. <https://doi.org/10.1590/S0074-02761984000300004>
- Guimarães AE, Mello RP, Lopes CM, Alencar J, Gentile C. 1997. Prevalência de Anofelinos (Diptera: Culicidae) no crepúsculo vespertino em áreas da Usina Hidrelétrica de Itaipu, no município de Guairá, estado do Paraná, Brasil. *Memórias do Instituto Oswaldo Cruz*. 92:745-754. <https://doi.org/10.1590/S0074-02761997000600004>
- Guimarães AE, Mello RP, Lopes CM, Gentile C 2000. Ecology of mosquitoes (Diptera: Culicidae) in areas of Serra do Mar State Park, State of São Paulo, Brazil. I- Monthly frequency and climatic fac-

- tors. *Memórias do Instituto Oswaldo Cruz*. 95:1-16. <http://dx.doi.org/10.1590/S0074-02762000000600002>
- Harbach RE. 2020. Mosquito Taxonomic Inventory. Culicidae Classification. Natural History Museum, London, UK. Available from URL: <http://mosquito-taxonomic-inventory.info/simpletaxonomy/term/6045> (Accessed 26 October 2020)
- IBGE. 1988. Geografia do Brasil, Região Centro-Oeste. Vol I., Rio de Janeiro: Fundação Instituto Brasileiro de Geografia e Estatística. 268 pp.
- Iversson LB, Silva RAMS, Travassos da Rosa APA, Barros VLRS. 1993. Circulation of eastern equine encephalitis, western equine encephalitis, ilheus, maguari and tacaiuma viruses in equines of the Brazilian Pantanal, South America. *Revista do Instituto de Medicina Tropical de Sao Paulo*. 35:355-359. <https://doi.org/10.1590/S0036-46651993000400009>
- Junk WJ, Bayley PB, Sparks RS. 1989. The flood pulse concept in river - floodplain systems. Pp. 110-127. D.P. Dodge (ed.). In: Proceedings of the International Large River Symposium (LARS). *Canadian Journal of Fisheries and Aquatic Sciences*. 106:110-127.
- Lane J. 1953. *Neotropical Culicidae*. São Paulo: Universidade de São Paulo. 548 pp.
- Marques MI, Adis J, Santos GB, Battirola LD. 2006. Terrestrial arthropods from tree canopies in the Pantanal of Mato Grosso, Brazil. *Revista Brasileira de Entomologia*. 50:257-267. <https://doi.org/10.1590/S0085-56262006000200007>
- Martins JR, Medri IM, Oliveira CM, Guglielmone A. 2004. Ocorrência de carrapatos em tamandua bandeira (*Myrmecophaga tridactyla*) e tamanduá-mirim (*Tamandua tetradactyla*) na região do Pantanal Sul Mato-Grossense, Brasil. *Ciência Rural*. 34:293-295. <https://doi.org/10.1590/S0103-84782004000100048>
- Martins LMO, David MR, Maciel-de-Freitas R, Silva-do-Nascimento TF. 2018. Diversity of *Anopheles* mosquitoes from four landscapes in the highest endemic region of malaria transmission in Brazil. *Journal of Vector Ecology*. 43:235-244. <https://doi.org/10.1111/jvec.12307>
- Mauro RA. 1993. Dissertação Mestrado. Abundância e padrão de distribuição de cervo-do-pantanal *Blastocerus dichotomus* (Illiger, 1815), no Pantanal Mato-Grossense. Belo Horizonte: Universidade Federal de Minas Gerais, Brasil.
- Melandri V, Alencar J, Guimarães AE. 2015. The influence of the area of the Serra da Mesa Hydroelectric Plant, State of Goiás, on the frequency and diversity of anophelines (Diptera: Culicidae): a study on the effect of a reservoir. *Revista da Sociedade Brasileira de Medicina Tropical*. 48:33-38. <https://doi.org/10.1590/0037-8682-0225-2014>
- Melandri V, Guimarães AE, Komar N, Nogueira M, Mondini A, Alencar J, Bosch I. 2012. Serological detection of West Nile virus in horses and chicken from Pantanal, Brazil. *Memórias do Instituto Oswaldo Cruz*. 107:1073-1075. <https://doi.org/10.1590/S0074-02762012000800020>
- Moraes AS, Resende EK, Rodrigues CAG, Mauro RA, Galdino S, Oliveira MD, Crispim SAA, Vieira LM, Soriano BMA, Abreu UGP, Mourão GM. 2000. Embrapa Pantanal: 25 anos de pesquisas em prol da conservação do Pantanal. In: III Simpósio sobre recursos naturais e sócio-econômicos do Pantanal, Corumbá, Brasil. 55 pp.
- Natal D, Barata EAMF, Urbinatti PR, Barata JMS. 1995. Contribuição ao conhecimento da fauna de imaturos de mosquitos (Diptera: Culicidae) em área de implantação de hidrelétrica na bacia do rio Paraná, Brasil. *Revista Brasileira de Entomologia*. 39:897-899.
- Nunes AP, Tomas WM. 2008. Aves migratórias e nômades ocorrentes no Pantanal. Corumbá: Embrapa Pantanal. 124 pp. <https://ainfo.cnptia.embrapa.br/digital/bitstream/CPAP-2009-09/56881/1/Livro033.pdf>
- Nunes da Cunha C, Junk WJ. 2004. Year-to-year changes in water level drive the invasion of *Vochysia divergens* in Pantanal grasslands. *Applied Vegetation Science*. 7:103-110. <https://doi.org/10.1111/j.1654-109X.2004.tb00600.x>
- Oliveira-Ferreira J, Lourenço-de-Oliveira R, Teva A, Deane LM, Daniel-Ribeiro CT. 1990. Natural malaria infections in anophelines in Rondônia state, Brazilian Amazon. *American Journal of Tropical Medicine and Hygiene*. 43:6-10. <https://doi.org/10.4269/ajtmh.1990.43.1.TM0430010006>
- Póvoa MM, Wirtz RA, Lacerda RNL, Miles MA, Deane LM, Daniel-Ribeiro CT. 2001. Malaria vectors in the municipality of Serra do Navio, State of Amapá, Amazon Region, Brazil. *Memórias do Instituto Oswaldo Cruz*. 96:179-184. <http://dx.doi.org/10.1590/S0074-02762001000200008>

- Quintero LO, Thatcher BD, Tadei P. 1996. Biologia de anofelinos amazônicos. XXI. Ocorrência de espécies de *Anopheles* e outros culicídeos na área de influência da hidrelétrica de Balbina - cinco anos após o enchimento do reservatório. *Acta Amazônica*. 26:281-296. <https://doi.org/10.1590/1809-43921996264296>
- Ravazzani C, Wiederkehr Filho H, Fagnani JP, Costa S. 1990. Pantanal. Curitiba: Editora Edibran. 181 pp.
- Reinert JF. 2001. Revised list of abbreviations for genera and subgenera of Culicidae (Diptera) and notes on generic and subgeneric changes. *Journal of the American Mosquito Control Association*. 17:51-55.
- Silva-Vasconcelos A, Kató MYN, Mourão EM, Souza RTL, Lacerda RNL, Sibajev A, Tsiuris P, Povia MM, Momen H, Rosa-Freitas MG. 2002. Biting indices, hostseeking activity and natural infection rates of Anopheline species in Boa Vista, Roraima, Brazil from 1996 to 1998. *Memórias do Instituto Oswaldo Cruz*. 97:151-161. <http://dx.doi.org/10.1590/S0074-02762002000200002>
- Tadei WP, Costa WLS. 1985. Estudos das espécies de *Anopheles* da área da hidrelétrica de Samuel (Rondônia). *Ciências e Cultura*. 37:751.
- Tadei WP, Thatcher BD. 2000. Malaria vectors in the Brazilian Amazon: *Anopheles* of the the subgenus *Nyssorhynchus*. *Revista do Instituto de Medicina Tropical, São Paulo*. 42:87-94. <http://dx.doi.org/10.1590/S0036-46652000000200005>
- Teodoro U, Guilherme ALF, Lozovei AL, Salvia Filho V, Fukushigue Y, Spinosa RP, Ferreira MEMC, Barbosa OC, Lima EM. 1995. Culicídeos do lago de Itaipu no rio Paraná, sul do Brasil. *Revista de Saúde Pública*. 29:6-14. <http://dx.doi.org/10.1590/S0034-89101995000100003>
- Vittor AY, Gilman RH, Tielsch J, Glass G, Shields T, Lozano WS, Pinedo-Cancino V, Patz JA. 2006. The effect of deforestation on the human-biting rate of *Anopheles darlingi*, the primary vector of falciparum malaria in the Peruvian Amazon. *American Journal of Tropical Medicine and Hygiene*. 74:3-11. <https://doi.org/10.4269/ajtmh.2006.74.3>
- Ward RA. 1984. Second supplement to "A catalog of the mosquitoes of the world" (Diptera: Culicidae). *Mosquito Systematics*. 16:227-270.
- Ward MP. 2005. Epidemic West Nile virus encephalomyelitis: A temperature-dependent, spatial model of disease dynamics. *Preventive Veterinary Medicine*. 71:253-264. <https://doi.org/10.1016/j.prevetmed.2005.07.008>
- World Health Organization. 2018. World health statistics 2018: monitoring health for the SDGs, sustainable development goals. *World Health Organization*. VIII + 86 pp. <https://apps.who.int/iris/handle/10665/272596>.