



Diversity and spatio-temporal variation of *Anopheles* (Diptera: Culicidae) before and after the construction of the Jirau hydroelectric plant, state of Rondônia, Brazil

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ABSTRACT

The objective of this study was to evaluate the effect of the construction of the Jirau hydroelectric plant on the anopheline species, in Porto Velho, Rondônia, Brazil. For this, sampling was carried out in 23 locations in the pre (2004) and 23 after construction (2018) phases in the hydroelectric dam's coverage area, performed by human landing catch between 6:00 pm and 10:00 pm. We found 14 anopheline species. In the pre-construction phase, *An. darlingi* (73%) was the most abundant and after construction *An. braziliensis* (31.5%) was the most abundant. The T test indicated that there were no differences in the indexes – diversity (H'), richness (Sjack 1), equitability (J') and dominance (d) of anophelines species - evaluated between the phases ($p > 0.05$). The spatio-temporal distribution of *An. darlingi* proved to be wide, with the highest density values observed in March ($\bar{x}_w = 27.1$) in the pre-construction phase and in the months of May ($\bar{x}_w = 4$) and July ($\bar{x}_w = 3.1$), post-construction. Differences in hematophagous activity peaks were observed between species and between phases. According to the data obtained in this study, the construction of the hydroelectric plant had little influence on the composition of anopheline species, however changes were observed in the spatio-temporal distribution of the *An. darlingi* and in the pattern of hematophagous activity of the species, which directly influenced the dynamics of malaria in the region.

Introduction

After the acceleration of the industrialization process, the economy started to explore the energy potential in water resources to meet the national demand (Sadorsky, 2013; Li and Lin, 2015). Therefore, during the 70s the construction of hydroelectric plants began in the Brazilian Amazon region, being Coaracy Nunes in the state of Amapá, Curuá-Una and Tucuruí in the state of Pará and Balbina in Amazonas, in addition to these, the Brokopondo in Suriname. The region has the largest hydrographic basin in the world and has a high potential for energy production (Fearnside, 2015; Tolmasquim, 2016).

The installation of hydroelectric projects implies changes in the environment with possible impacts on flora and fauna (Odinetz-Collart, 1987; von Sperling, 2012). In addition, another relevant aspect is the increase in the incidence of pathogen transmitted by insect vectors, of water transmission and others related to migration (Tadei et al., 2017). In this context, diseases such as arboviruses, schistosomiasis, filariasis and malaria were frequently registered in areas covered by hydroelectric plants in the Brazilian Amazon (Junk and Nunes de Mello, 1987, 1990; Couto, 1999; Sanchez-Ribas et al., 2012).

The malaria is considered a serious public health problem worldwide and in Brazil ~ 99.8% of cases are registered in the Brazilian Amazon region, where approximately 42.5 million people live in risk areas

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(Oliveira-Ferreira et al., 2010; Ferreira and Castro, 2016; WHO, 2019). The maintenance of this disease is directly associated with human action in the environment, characterized by disordered occupations in urban and peri-urban spaces, deforestation, irrigation projects, construction of fish farming tanks, exploration of fossil fuels, minerals and natural gas and construction of highways, greatly contributing to the exposure of man to the vector and consequently to the pathogen transmission (Tadei et al., 1988, 1998, 2017; Padilha et al., 2019; Chaves et al., 2020). In addition, climatic factors can favor transmission, such as el niño and la niña and global climate changes that promote variations in precipitation, humidity, temperature and level of rivers in the region (Wolfarth-Couto et al., 2019).

According to Tadei et al. (2017), environmental changes in malaria endemic regions result in changes in the degree of disease incidence. This fact is directly associated with the increase in the density of *Anopheles darlingi* Root, 1926, the main vector of human malaria in Brazil. This mosquito can trigger outbreaks of the disease even in low density situations due to the anthropophilic behavior (Tadei et al., 1993; Tadei and Dutary-Thatcher, 2000; Sallum et al., 2020). In addition, other species may act in the transmission of *Plasmodium* in the region, among which are *Anopheles aquasalis* Curry, 1932, *Anopheles albicans* s.l Lynch Arribálzaga, 1878, *Anopheles triannulatus* (Neiva and Pinto, 1922), *Anopheles nuneztovari* Galbadon, 1940 and *Anopheles oswaldoi* (Peryassú, 1922) (Tadei et al., 1993; Deane, 1986).

Another relevant factor from the epidemiological point of view in areas covered by hydroelectric plants is the increase in the migratory flow of human populations attracted by employment opportunities, a fact observed in hydroelectric dams in the state of Pará - Tucuruí and Belo Monte - in addition to the Santo Antônio and Jirau hydroelectric dams in the state of Rondônia (Fearnside, 1999, 2001; Barreto et al., 2011). In general, hydroelectric projects can offer risks regarding the increase in the number of malaria cases, because in addition to perening anophelines breeding sites on the banks of reservoirs, a fact that contributes to maintaining high densities of vectors throughout the year, human populations often come from non-endemic regions and have greater susceptibility to infection by *Plasmodium* species, when compared to Amazonian populations (Katsuragawa et al., 2009). In addition, the entry of oligosymptomatic individuals in these areas is also emphasized, introducing the etiological agent and favoring outbreaks and maintenance of malaria (Tadei et al., 1983, 1998).

The Brazilian environmental legislation, aiming to mitigate these impacts, determined that the enterprises subject to environmental licensing in the Amazon must comply with the provisions of Ordinance of the Ministry of Health No. 47 of 12/29/2006 (MS, 2006), this being then revoked by Ordinance of the Ministry of Health No. 01 of 01/13/2014 (MS, 2014). This legal provision obliges enterprises to implement an Action Plan for Malaria Control - PACM, in line with the National Malaria Prevention and Control Program - PNCM (MS, 2003), aimed at workers' health and the communities located in the area covered by these projects. PACMs consist of protocols of intentions where enterprises are required to adopt exclusive mitigation and compensatory measures for the control of malaria with the municipalities in the coverage area where they are installed. In this way, public-private partnerships are established where both (municipality and entrepreneur) have defined roles in the responsibilities and commitments to the reduction of malaria (MS, 2014). The main result of the implementation of PACMs in Porto Velho was that the municipality managed to reduce the number of cases of the disease by 83%, a fact that led to the evolution of the level of high risk of transmission in 2010 to low risk since 2015 (SEMUSA-PMPV, 2017).

Some species of mosquitoes can be favored from the environmental changes resulting from the construction of hydroelectric plants, as the artificial lake becomes a breeding ground for several culicids as

observed in the Tucuruí, Balbina, Samuel, Itaipu and Porto Primavera hydroelectric plants, where there was a significant increase in frequency of anophelines in the post-construction stages of the project (Tadei et al., 1983; Osório-Quintero et al., 1996; Tadei et al., 1998; Paula and Gomes, 2007; Silva et al., 2010; Paula et al., 2012; Rodrigues et al., 2017; Pires et al., 2019; Rêgo et al., 2020). These mosquitoes in natural conditions develop in breeding sites consisting of black water floodplain forests (igapó), backwaters of rivers and streams (Arcos et al., 2018). However, adaptations to anthropized environments have enabled proliferation of anophelines in artificial breeding sites, such as fish ponds, pottery pools and hydroelectric ponds (Guimarães et al., 2004; Ferreira et al., 2015).

The area covered by hydroelectric dams on the Madeira River in Rondônia has been the subject of studies with the objective of understanding aspects related to the bioecology of anopheline species, with high density records by *An. darlingi* (Cruz et al., 2009; Morais et al., 2012; Gil et al., 2015; Rodrigues et al., 2017). The study of the diversity and distribution of anophelines in endemic areas that have undergone some process of environmental change is of great epidemiological value, as there are variable and specific conditions for each location, considering the complexity of the Amazon ecosystem (Tadei et al., 1983).

Therefore, the objective of this study was to analyze aspects related to diversity, spatial and temporal distribution and behavioral patterns of anophelines during the pre and post-construction phases of the Jirau hydroelectric plant, and the possible epidemiological implications.

Methods

Study area

The state of Rondônia has an important position in relation to phytogeographic aspects, being located in the center of the Amazon basin, in a transition region between the geomorphological domain of Central Brazil and the Amazon geomorphological domain. The municipality of Porto Velho, the capital of state, is located in the north of the state, with a territorial area of 34.082.37 km² and a population of 539.354 inhabitants, with a demographic density of 12.57 inhabitants/km² and its rural population is 37.794 (IBGE, 2019). Porto Velho's climate is of the Am type, according to the Köppen classification, consisting of a humid tropical climate, with a dry media capacity of less than 10 mm and an annual media capacity of 2.355 mm. The vegetation of the area is selected as an open tropical forest, with a predominance of red-yellow latosol. The Jirau hydroelectric plant was a work of the PAC - Growth Acceleration Program of the Federal Government of Brazil. The dam is located on the axis where the Ilha do Padre was located, 120 km away from the headquarters of the municipality of Porto Velho, with formation of a flooded area in 2013 and estimated useful life of more than 50 years, with dynamic level, that is, the level of the lake follows the dynamics of the flows of rivers in the Amazon. The production of energy occurs through the use of 50 turbines inserted in the waterway of the Madeira River, with a reservoir of 361.6 km² and an installed capacity to generate 3.750 MW, sufficient to supply 10 million homes (ESBR, 2020).

Sampling was carried out at different times covering the pre-construction and post-construction phases of the hydroelectric plant. In the pre-construction phase, monthly collections were carried out from January to August 2004, in 23 locations and after construction also in 23 locations from March to October 2018 (Fig. 1) (license number SISBIO n°58855-1). The section studied comprises the districts of Jaci Paraná and Abunã, including the Abunã ferry, performing a linear distance of 138 km. The area studied comprises areas close to the main bed of the Madeira River and points located in vicinal areas located on the right bank of BR 364 Highway. The criterion used for the

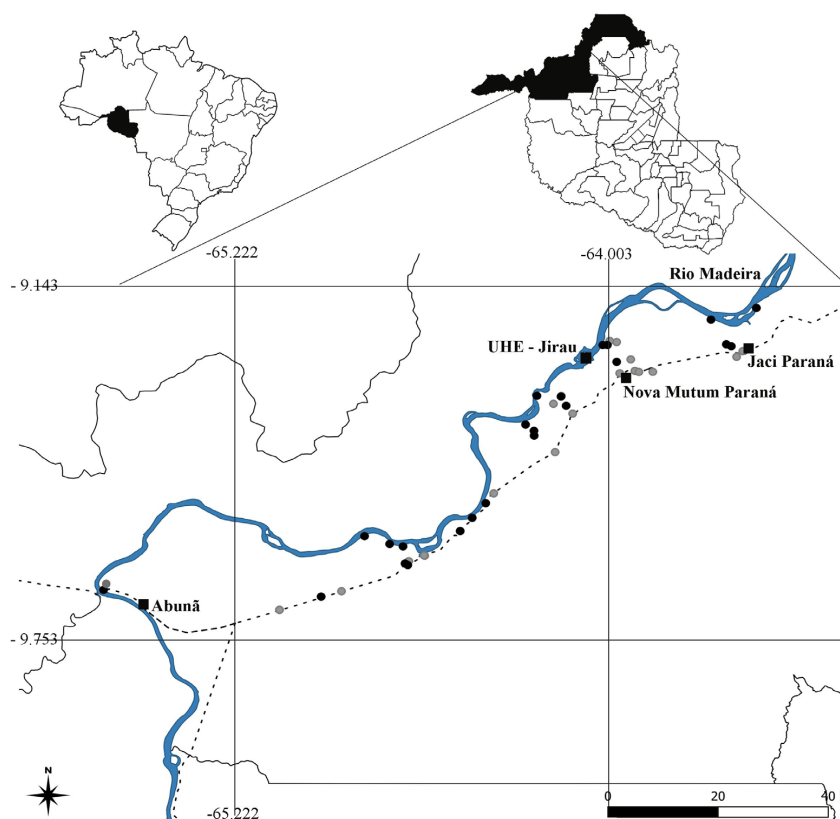


Figure 1 Sampling points of anophelines in the area covered by the Jirau hydroelectric plant, in the stretch between the locations of Jaci Paraná and Abunã (squares), in the pre (black) and post-construction (gray) phases.

selection of the collection points was the presence of human dwellings in situations of risk for malaria that were in the area covered by the Jirau hydroelectric plant.

Sampling of mosquitoes

The collections were performed by three collectors in a non-rotated performed by Human Lading Catch (HCL) (INPA Ethics Committee N°3.474.088) in the peridomiciliary area from human dwelling, with an effort of 344 collection hours in each evaluated phase. In the pre and post-construction phases, sampling was carried out between 6:00 pm and 10:00 pm. In this way, mosquitoes attracted by the human odor (cairomonium) were aspirated and transferred to paraffin cups with the aid of Castro's captor, and in followed transported to the Malaria and Dengue Laboratory, of the Instituto Nacional de Pesquisas da Amazônia - INPA. The capture activities were conducted as determined by the Ministry of Health of Brazil (MS, 2019), so everyone involved was wearing personal protective equipment consisting of black socks, long jeans, long shirts, closed shoes and a hat.

At the laboratory, the specimens were killed in a freezer (-20 °C), labeled and stored for later identification. The identification was carried out with the aid of keys proposed by Goham et al. (1967), Consoli and Lourenço de Oliveira (1994) and Faran and Lintichum (1981). Due to *An. triannulatus*, *An. albitarsis*, *An. oswaldoi* and *An. nuneztovari* to represent species complexes, in this study these species were called sensu lato. The specimens are deposited in the collection of the Laboratório de Malária e Dengue; later, a part them will be sent to the Collection of Invertebrates of the Instituto Nacional de Pesquisas da Amazônia, INPA.

Data analysis

The relative abundance (%) of anopheline species was calculated for each phase studied. The punctual data of *An. darlingi* in the sampled area during the pre and post-construction phases were used to evaluate the spatial distribution of this species in the section between the districts of Jaci Paraná (BR 364 Federal Highway, km 799) and Abunã (BR 364 Federal Highway, km 937). For that, a heat map was obtained using the Kernel method with the aid of the Qgis software v.3.4.12. In addition, Shannon's diversity indexes (H'), richness (1st order Sjack), Piloni equitability (J') and Berger-Parker dominance (d') were calculated using the software DivEs - Diversidade de Espécies @ v. 4.15 (available in <https://dives.ebras.bio.br/>), for both phases (pre and post-construction) (Rodrigues 2017). In order to assess possible changes, the values of these indices were compared using the T test ($p < 0.05$).

It was also evaluated the pattern of greater hematophagic activity of the most abundant species in the studied phases. We consider the following intervals: from 6:00 pm to 7:00 pm, 7:00 pm to 8:00 pm, 8:00 pm to 9:00 pm, and 9:00 p.m. to 10:00 p.m. in the pre and post-construction phases. The specimens were organized in paraffin cups that were properly labeled, with information on the time interval for capture and location. The glasses were changed every 30 minutes, for a total time of 4 hours.

In order to assess the temporal distribution of the species, density was calculated using the Williams average (\bar{x}_w), used to demonstrate seasonal patterns and obtained using the following formula: $\bar{x}_w = [\text{antilog}(\sum \log(n+1) / N) - 1]$, where: \bar{x}_w : Williams average; n: number of individuals caught in the month; N: total number of months sampled (Forattini, 2002).

The monthly averages of temperature ($^{\circ}\text{C}$), rainfall (mm) and relative humidity (R. H. %) were obtained from the Instituto Nacional de Meteorologia of Brazil (INMET) and pluviometric data, available on the website of the Agência Nacional de Águas of Brazil (ANA). Malaria data were obtained through SIVEP / SVS (Sistema de Informação de Vigilância Epidemiológica de Secretaria de Vigilância em Saúde – SVS of Brazil) from the following locations in the area covered by the Jirau hydroelectric plant: Abunã and Jaci Paraná. In order to assess the possible relationships between *Anopheles* species, environmental variables and the number of cases of malaria, they were subjected to canonical correlation analysis (CCA), for each sampled phase. All statistical analyzes were calculated with the aid of the PAST v. 2.17 (PALEontological Statistics) software (available in https://palaeo-electronica.org/2001_1/past/issue1_01.htm).

Results

Species composition

The anopheline fauna found in the area covered by the Jirau hydroelectric plant, considering the pre- and post-construction phases, was composed of 14 species, distributed in the subgenera *Anopheles* and *Nyssorhynchus*. In the pre-construction phase, 12 species were found and *An. darlingi* was the most abundant (73%), followed by *An. albitarsis* (12.9%). The least abundant were *An. rangeli* and *An. mattogrossensis* (both with 0.03%) (Table 1). In the post-construction phase, *An. braziliensis* (31.5%) was the most abundant species out of the total of 13 species found, followed by *An. darlingi* (28%) and the least abundant were *An. rangeli* and *An. mediopunctatus* (both with 0.05%).

Ecological indexes

The diversity (H') of anophelines in the pre-construction phase of the hydroelectric plant varied between 0.5719 and 1.5294 and the values of richness (Sjack 1) between 3 and 10, with an average of 6.875. The equitability values (J') varied between 0.2213 and 0.9649, while dominance (d) varied between 0.4083 and 0.92. According to the values of dominance (d), *An. darlingi* was dominant in all samples.

In the post-construction phase, the diversity values (H') varied between 0.7133 and 2.2153. The values of richness (Sjack 1) varied

between 4 and 12, with an average of 6.62, slightly lower than that found in the pre-construction phase, while those of equitability (J') varied between 0.3072 and 0.7629. Considering the dominance data (d), *An. darlingi* was dominant in 4 of the 8 samplings performed and in the other months the dominant species were: *An. braziliensis*, *An. nuneztovari* and *An. albitarsis*. The T test revealed that there were no significant differences ($p > 0.05$) between the variables - diversity, richness, equitability and dominance - between the pre and post-construction phases of the Jirau hydroelectric plant. However, changes in dominance were observed, since in the pre-construction phase *An. darlingi* was the dominant species in all samples, while in the post-construction phase there was alternation of dominance between *An. darlingi* and other *Anopheles* species.

Spatio-temporal distribution of *Anopheles darlingi*

In the area covered by the Jirau hydroelectric plant, it was possible to sample the homes around houses of different characteristics: 2a - house located on a site in a forest area; 2b - wooden house in varzea area with straw roof; 2c - brick house in a village with clay roof and 2d - wooden house with asbestos tile. The difference observed in the abundance of *An. darlingi* between the phases was reflected in the heat map obtained from the Kernel method. Differences were observed in the spatial distribution of *An. darlingi* where in the pre-construction phase a high abundance of this species was observed along the stretch between Jaci Paraná and Mutum Paraná (Fig. 2e), especially in the vicinity of the localities of Jaci Paraná. On the other hand, in the post-construction phase, a reduction in areas of high abundance was observed, registering only in the region of near the Jirau hydroelectric plant (Fig. 2f).

According to the Williams average (\bar{x}_w) obtained in the pre-construction phase, *An. darlingi* showed a higher density in March ($\bar{x}_w = 27.1$), with a sharp drop in the subsequent months, thus presenting a unimodal pattern of temporal distribution. The averages observed for *An. albitarsis* and *An. braziliensis* did not show great variation, remaining low during the sampled phase (Fig. 3a). A bimodal pattern of temporal distribution of *An. darlingi* was observed in the post-construction phase, in May ($\bar{x}_w = 4$) and July ($\bar{x}_w = 3.1$), respectively. The highest values of density of *An. nuneztovari* and *An. braziliensis* were observed in March ($\bar{x}_w = 0.8$) and June ($\bar{x}_w = 1.18$), respectively. It is noteworthy that the

Table 1
Relative abundance (%) of anophelines collected in the pre (2004) and post construction (2018) phases of the Jirau hydroelectric plant, in Rondônia, Brazil.

Subgenera	Species	Pre		Post	
		N	R. A (%)*	N	R. A (%)*
<i>Anopheles</i>	<i>Anopheles peryassui</i> Dyar & Knab, 1908	35	1.35	6	0.35
	<i>Anopheles mattogrossensis</i> Lutz & Neiva, 1911	1	0.03	11	0.5
	<i>Anopheles mediopunctatus</i> (Lutz, 1903)	8	0.30	1	0.05
	<i>Anopheles minor</i> da Costa Lima, 1929	-	-	14	0.9
<i>Nyssorhynchus</i>	<i>Anopheles albitarsis</i> s.l Lynch Arribálzaga, 1878	337	12.9	135	9
	<i>Anopheles argyritarsis</i> Robineau-Desvoidy, 1827	-	-	2	0.15
	<i>Anopheles benarrochi</i> Gabaldon, Cova & Lopez, 1941	2	0.07	-	-
	<i>Anopheles braziliensis</i> (Chagas, 1907)	241	9.2	476	31.5
	<i>Anopheles darlingi</i> Root, 1926	1897	73	426	28
	<i>Anopheles evansae</i> (Brèthes 1926)	4	0.15	32	2.1
	<i>Anopheles nuneztovari</i> Gabaldon, 1940	14	0.55	254	17
	<i>Anopheles oswaldoi</i> s.l (Peryassú 1922)	9	0.35	6	0.4
	<i>Anopheles rangeli</i> Gabaldon, Cova-Garcia & Lopez, 1940	1	0.03	1	0.05
	<i>Anopheles triannulatus</i> s.l (Neiva & Pinto, 1922)	43	2	141	9.5
<i>Anopheles</i> sp.	2	0.07	8	0.5	
Total	2594	100	1513	100	

*R. A – Relative Abundance

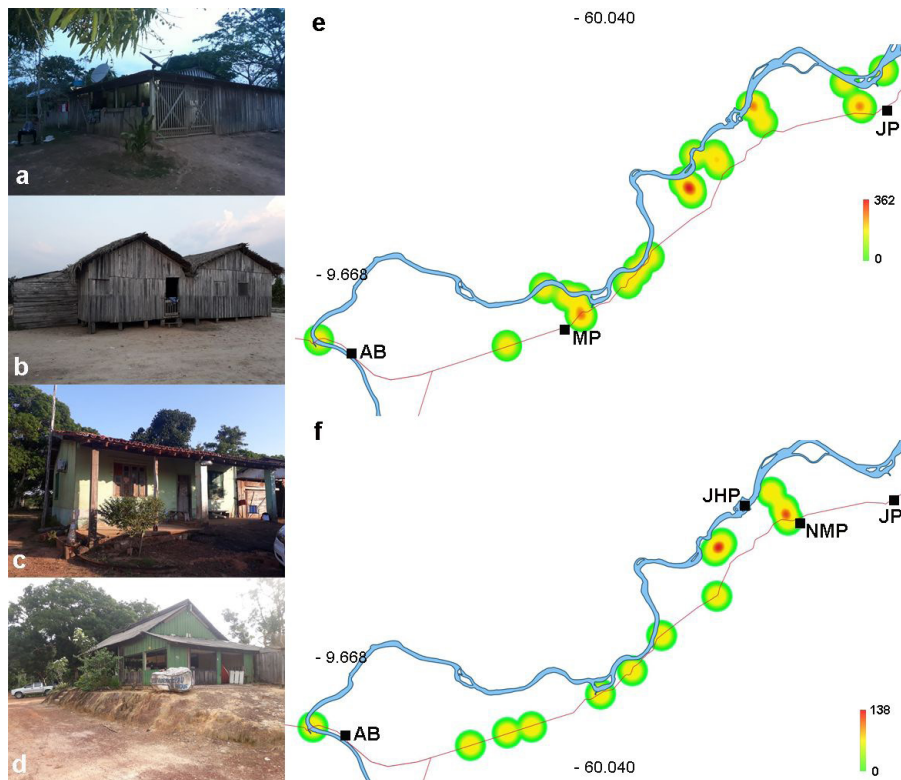


Figure 2 Housing types (a-d) spatial variation of *Anopheles darlingi* before (e) and after (f) the construction of the Jirau hydroelectric plant, in Rondônia, Brazil. Subtitle: AB – Abunã; JHP – Jirau Hydroelectric Plant; JP – Jaci Paranã; NMP – Nova Mutum Paranã.

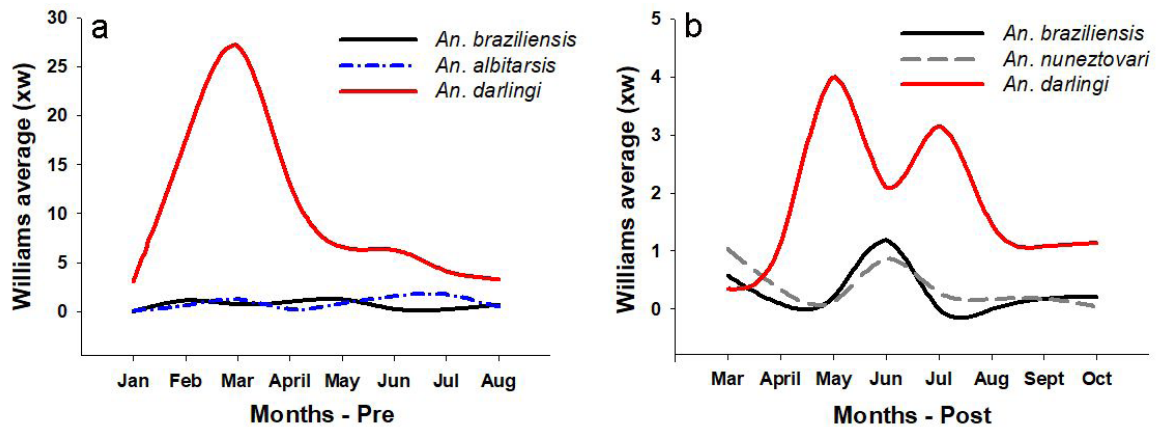


Figure 3 Density of *Anopheles* species (\bar{x}_w) in the sampled months (January to August) before (a) and after (March to October) the construction (b) of the Jirau hydroelectric plant, in Rondônia, Brazil.

average Williams (\bar{x}_w) of the species were lower than those found in the pre-construction phase of the Jirau hydroelectric plant (Fig. 3b).

Patterns of hematophagic activity

In the pre-construction phase of the hydroelectric plant, it was observed that the interval of greatest hematophagic activity was between 7:00 pm and 8:00 pm. The hematophagic activity of *An. darlingi* was intense in all the hourly intervals analyzed, mainly between 7:00 pm and 8:00 pm, in which 50.3% of the individuals of this species were observed, in this interval the peak of activity was observed for the other species evaluated (*An. albitarsis* – 41.5%, *An. braziliensis* – 57.6%), except *An. peryassui*, greater between 6:00 pm and 7 pm. After this interval, between 9:00 pm and 10:00 pm, a decrease in the hematophagic activity of all analyzed species was observed (Fig. 4a). In the post-construction

phase, it was observed that the peak of greater hematophagic activity was between 6:00 pm and 7:00 pm, observed pattern for *An. darlingi* (52.3%), *An. albitarsis* (80.7%) and *An. nuneztovari* (74%). This pattern was different from that observed for *An. braziliensis* and *An. triannulatus*, both were more frequent in the interval between 7:00 pm and 8:00 pm (58.8% and 78.7%, respectively). After 9:00 pm, a drop in hematophagic activity was observed for all analyzed species (Fig. 4b).

Environmental characteristics and species of *Anopheles*

In the pre-construction phase, the temperature values (°C) varied between 14.3 and 35.4, the relative humidity (%) varied between 71 and 85 while the precipitation (mm) between 7.4 and 339. In the post-construction phase, the temperature (°C) varied between 23.8 and 26.3, the relative humidity of the air (R. H. %) varied between 73 and 89.8, while

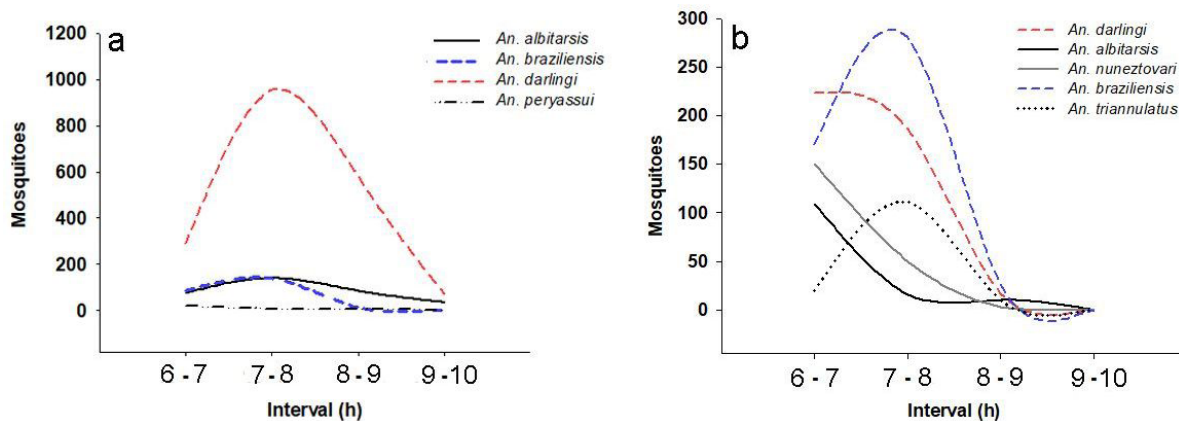


Figure 4 Hematophagic activity patterns of anophelines in the pre (a) and post-construction (b) phases of the Jirau hydroelectric plant, in Rondônia, Brazil.

the precipitation (mm) varied between 1.2 and 343.6. The fluviometric data (cm) indicated that the river level of the Madeira River varied between 877 and 1451 in the pre-construction phase and between 836 and 1684, in the post-construction phase. It was observed that canonical analyzes 1 and 2 were the most informative in both phases, explaining 70.6% and 98.6% of the influence of environmental factors on *Anopheles* species, respectively (Figs. 5a, 5b). This analysis revealed that the *An. peryassui*, *An. nuneztovari*, *An. darlingi* and *An. triannulatus* showed to be correlated ($r^2 > 0.5$) with the relative humidity of the air (R. H%), in the pre-construction phase. The last two were also correlated ($r^2 > 0.5$) with atmospheric temperature ($^{\circ}\text{C}$). The number of malaria cases was strongly correlated ($r^2 > 0.6$) with *An. darlingi*, *An. evansae* and *An. nuneztovari*. The species *An. darlingi*, *An. nuneztovari* and *An. triannulatus* are also shown to be correlated with the river level at this stage (Fig. 5a).

In the post-construction phase, *An. mattogrossensis*, *An. triannulatus* and *An. evansae* showed a correlation ($r^2 > 0.5$) with the river level. Only *An. albitarsis* correlated with atmospheric temperature ($^{\circ}\text{C}$), while *An. braziliensis*, *An. minor* and *An. argyritarsis* demonstrated positive correlation ($r^2 > 0.4$) with rainfall (mm^3). At this phase, the number of cases of malaria showed a positive correlation with *An. oswaldoi* ($r^2 > 0.5$) (Fig. 5b). Although the number of cases of malaria did not correlate with *An. darlingi* in the post-construction phase, it is possible to observe in Fig. 6 that the density of this species is accompanied by the incidence of cases of malaria in both phases studied. According to data from SIVEP (Epidemiological Surveillance System/Ministry of Health of Brazil) in 2004, a total of 4.415 cases of malaria were reported, adding up to the total number of cases recorded in the locations of Abunã and Jaci Paraná. However, in 2018, 21 cases were reported in these same locations.

Discussion

Previous studies cite six species of anophelines frequently associated with malaria transmission in the Brazilian Amazon: *An. albitarsis*, *An. aquasalis*, *An. braziliensis*, 1908, *An. darlingi*, *An. nuneztovari* and *An. triannulatus*. Of these, only *An. aquasalis* was not obtained in this study because it is not endemic to the western Brazilian Amazon region (Tadei and Dutary-Thatcher, 2000; Alimi et al., 2015; Laporta et al., 2015; Bourke et al., 2018). It was observed that *An. darlingi* was the most abundant species in both phases analyzed (Table 1), corroborating the data obtained by Morais et al. (2012), Gil et al. (2015) and Rodrigues et al. (2017), when they investigated the anopheline fauna in Porto Velho (RO). Another species that also showed high abundance in both phases was *An. braziliensis*, corroborating with other studies carried out in the Amazon that also found a high abundance of this species (Barbosa et al., 2016).

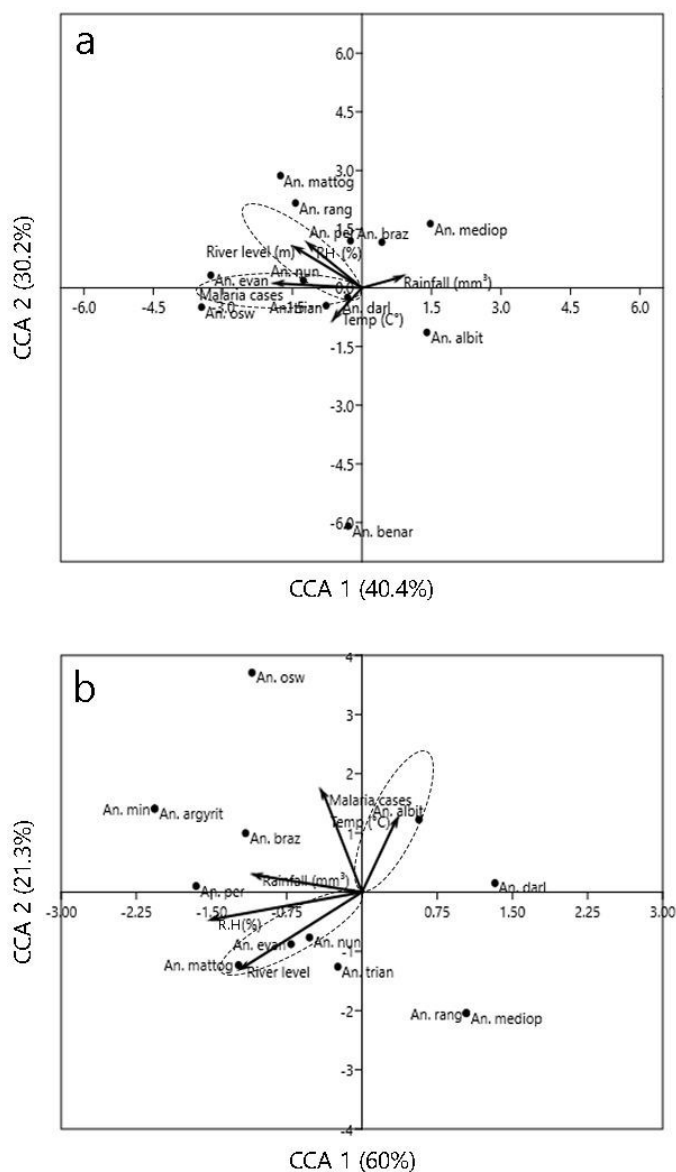


Figure 5 Canonical correlation analysis (CCA) ordering diagram between environmental factors and *Anopheles* species in the pre (a) and post-construction (b) phases of the Jirau hydroelectric plant: Relative Humidity of the air (R. H%); Temp (Temperature $^{\circ}\text{C}$); Subtitle: Anopheles albit – *An. albitarsis*; Anopheles argyrit – *An. argyritarsis*; Anopheles benar – *An. benarrochi*; Anopheles braz – *An. braziliensis*; Anopheles darl – *An. darlingi*; Anopheles evan – *An. evansae*; Anopheles mattog – *An. mattogrossensis*; Anopheles mediop – *An. mediopunctatus*; Anopheles osw – *An. oswaldoi*; Anopheles per – *An. peryassui*; Anopheles rang – *An. rangeli*; Anopheles trian – *An. triannulatus*.

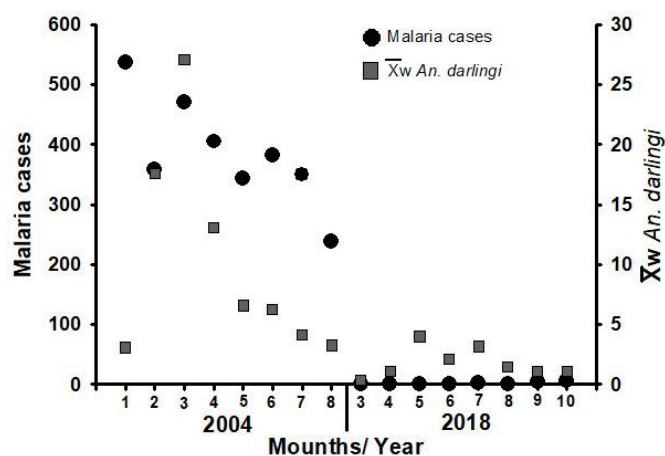


Figure 6 Cases of malaria and density of *Anopheles darlingi* in the area covered by the Jirau hydroelectric plant, in Rondônia, Brazil in the pre and post-construction phases. Source: SVS / SIVEP - Malaria.

Changes in the composition of anophelines fauna were observed between the pre and post-construction phases, such as the occurrence of *An. benarrochi* collected in the pre-construction phase and not collected in the post-construction phase. In addition, *An. argyritarsis* was only found after construction. According to Sallum et al. (1997), *An. benarrochi* was recorded in an area of intense agricultural activity related to sugar cane and pasture, so it is possible that the urbanization process that occurred in the area did not favor the permanence of this species in the environment.

Changes in the fauna composition were also observed in studies carried out in different stages of the construction of the Tucuruí hydroelectric plant, in the state of Pará (Tadei et al., 1998), highlighting the appearance of *An. argyritarsis* after the start of construction, a fact was also observed by Morais et al. (2012), evaluating the composition and richness of species in the coverage area of the Santo Antônio hydroelectric plant, in Rondônia. According to Silva et al. (2008), this species develops in artificial breeding sites and cohabits with *Aedes albopictus* (Skuse, 1894) in water tanks at ground level, showing itself adapted to anthropized environments. Therefore, it is possible that the environmental changes that occurred after the construction of the hydroelectric power plant favored the appearance of this species in the area.

According to the data obtained in this study, it was possible to observe the reduction in the abundance of *An. darlingi* in the post-construction phase of the Jirau hydroelectric plant (Table 1; Figs. 2e, 2f). These data differ from those found by Rodrigues et al. (2017). The authors analyzed the abundance of *An. darlingi* in the peridomicile of residences present in the area covered by the same enterprise, where they did not observe a reduction in the abundance of this species, between 2011 and 2015. Nevertheless, differences in anopheline abundance have been reported by other authors in areas covered by hydroelectric projects during the post-construction phase. These results were also similar to those found by Rezende et al. (2009), who did not observe an evolution in the abundance of anophelines after the construction of the Rosal hydroelectric plant, in southeastern Brazil, the authors highlighted that the increase in breeding availability was accompanied by deforestation that led to the scarcity of food for females, once that wildlife fauna has been suppressed.

We believe that the reduction in the abundance of *An. darlingi* in the coverage area after the construction of the hydroelectric plant is due to the population explosion of *Mansonia* (Diptera: Culicidae) that occurred in the region, after the historic flooding of the Madeira River in 2014. It is known that the females of this group of mosquitoes are voracious, aggressive and compete with the anophelines for blood sources at the same times (6 pm and 10 pm); in addition, these mosquitoes can still

bite in the daytime as long as the environmental conditions are adequate (Forattini, 2002; Navarro-Silva et al., 2004). Another factor that may have contributed to this reduced number was the implementation of intense control strategies by the private sector, acting on the potential breeding sites of *Anopheles* and performing thermo-fogging in areas with high density of *Mansonia* mosquitoes.

The evaluated indices - diversity (H'), richness (Sjackson 1), dominance (d) and equitability (J') did not show significant changes ($p < 0.05$) between the pre and post-construction phases, which is possibly due to few variations in the composition of species obtained in this study. In the post-construction phase, dominance was alternated between *An. braziliensis*, *An. albitarsis*, *An. nuneztovari* and *An. darlingi*. According to Tadei et al. (2017), the dominance of *An. darlingi* in anthropized environments occurs due to the highly anthropophilic behavior of this species (Tadei et al., 1993).

In this study, the high anthropophilia of *An. darlingi* had a direct influence on its spatial distribution, which was high around Jaci Paraná (18.131 inhabitants) and Nova Mutum Paraná (6.575 inhabitants), locations with the highest demographic indexes in the studied region, according to IBGE (2019) (Figs. 2e, 2f). We point out that the points of greatest abundance of *An. darlingi* in the post-construction phase were in the vicinity of the hydroelectric plant, this occurred due to the increase in the demographic density around the enterprise for economic reasons (Fig. 2f). Therefore, the data reinforce the anthropophilic behavior of this species when compared to other anophelines (Deane, 1986), with a close relationship with humans in the environment. These observations also corroborate those found by Gil et al. (2015), in a study on the seasonal distribution of anophelines in the rural area of Porto Velho, which observed a high density of *An. darlingi* close to the points of greatest demographic densities.

The temporal distribution of *An. darlingi* observed in this study indicated that March was the month that presented the highest value of density ($\bar{X}w$), during the pre-construction phase (Fig. 3a). A bimodal pattern of temporal distribution was observed in the post-construction phase, with two density peaks: in May and June (Fig. 3b). These data corroborate those found by Gil et al. (2015), these authors evaluated the seasonal distribution of anophelines in the rural area of Porto Velho and observed the occurrence of three peaks of *An. darlingi*, the first and second in March and May (end of the rainy season) and the third between August and September (end of dry season). The authors pointed out that after the construction of hydroelectric projects in the region, this third peak occurred in July.

In the Brazilian Amazon, the temporal distribution of anophelines populations varies according to the region due to the extensive territorial area and the high diversity of habitats that make up the biome. In the state of Amapá, located in the eastern Amazon, Barbosa et al. (2014) observed the unimodal temporal distribution of *An. darlingi*, differing from the data obtained in this study. The unimodal pattern of temporal distribution was also observed by Tadei and Dutary-Thatcher (2000), studying the anophelines in 15 locations in the Brazilian Amazon. They observed that on the AM 352 highway and in the municipality of Novo Airão (state of Amazonas), *An. darlingi* was more frequent in the month of June, that is, in the transition between the rainy and dry periods.

The most frequent species in the pre and post-construction phases of the Jirau hydroelectric plant showed a decrease in density in August, the hottest month of the year (Figs. 3a, 3b). This may be associated with a reduction in the availability of temporary breeding sites due to the low levels of precipitation and high temperatures, as a result of which there is a reduction in the population density of these mosquitoes and also in the transmission of malaria. According to Wolfarth-Couto et al. (2019, 2020), malaria cases are strongly correlated with rainfall and river levels, these factors increase the availability of breeding sites, in greater numbers in the rainy season and scarce at the height of the drought.

Concerning the pattern of hematophagic activity of the species, the first two hours after dark were preferred in both phases analyzed. Variation in the hematophagic activity of the species was observed between the pre-construction (from 7:00 pm to 8:00 pm) and post-construction (from 6:00 pm to 7:00 pm) phases (Figs. 4a, 4b), corroborating with the data observed by Tadei et al. (1998), who evaluated the activity of biting in four locations in the Amazon, where they observed that in three, including the municipality of Ariquemes, the time between 6:00 pm and 7:00 pm was the one with the greatest hematophagic activity of the females of *An. darlingi*, *An. nuneztovari*, *An. oswaldoi*, *An. triannulatus*, *An. albitarsis* and *An. braziliensis*. The change observed in the pattern of hematophagic activity of the species in this study corroborates the data found by Gomes et al. (2010), who observed differences in the pattern of hematophagic activity of *Anopheles* species after the implantation of the hydroelectric plant in the Paraná River. These authors also emphasize that these changes occurred due to the ecological changes that took place after the filling of the dam.

Malaria cases showed a positive correlation with *An. darlingi*, in the pre-construction phase and with *An. oswaldoi* in the post-construction ($r^2 > 0.5$) (Figs. 5 a, 5b). In this study, the high density of *An. darlingi* in the pre-construction phase coincides with the high number of malaria cases in 2004 in Jaci Paraná and Abunã, just as the drop in density of *An. darlingi* coincides with the reduced number of malaria cases in 2018, in the same locations, although the statistical analysis does not indicate a correlation, possibly due to the low number of registered cases (Fig. 6). In the post-construction phase, the number of malaria cases correlated with *An. oswaldoi* s.l. According to the data found by Branquinho et al. (1996) in Acre, this species was found naturally infected by *Plasmodium vivax* Grassi and Feletti, 1890, *Plasmodium vivax* V247, *Plasmodium falciparum* Welch, 1897, *Plasmodium malariae* Laveran, 1881, thus, it was considered the main vector of malaria in that region. Later, studies carried out by Marrelli et al. (1998, 1999), also investigated the susceptibility of this species and highlighted the relationship of this species with *P. vivax* in locations in the states of Acre and Rondônia.

According to Tadei et al. (1998), the peak density of *An. darlingi* is directly correlated with the period of greatest malaria transmission in the Amazon. Considering the environmental variables, a strong correlation was observed between *An. darlingi* and relative humidity of the air (R. H%) ($r^2 > 0.5$), differing from the data found by Barbosa et al. (2014), where this species showed no correlation with temperature and humidity, in a study on the composition and temporal variation of anophelines, in the state of Macapá, northern Brazil.

Several factors may have contributed to the decrease the cases of malaria between the pre and post-construction phases, in the area covered by the Jirau hydroelectric plant (Fig. 6). According to Padilha et al. (2019), this reduction can be attributed to socio-economic factors and loss of available habitats for deep-forest vectors (Laporta, 2019) as a result of deforestation in the region in recent years. In addition, we also emphasize the implementation of vector control actions by the government and also by the private sector through the Public Health Program for Energia Sustentável do Brasil - ESBR, related to the development of the following activities: a) vector control - application of adulticides, biolarvicides, installation of long-lasting impregnated mosquito nets; b) early diagnosis; c) identification of asymptomatic and d) education and health activities - workshops and lectures for the local population. These activities, together with the inspection of the public authorities, have a decisive role in malaria control in the area covered by the Jirau hydroelectric plant.

Conclusions

According to the results obtained in this study, the construction of the hydroelectric plant had little influence on the composition of anopheline

species where it was possible to observe small changes. However, we emphasize the changes were observed in the relative abundance, in the spatio-temporal distribution and in the pattern of hematophagic activity of the species that directly influenced the dynamics of malaria in the region.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

FAFSF and WPT contributed with experimental design, data analysis and manuscript writing; GRL and TMB contributed with the organism identification and data analysis; FMC, RAR, VMS, VF and VASN contributed with data analysis and manuscript review.

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