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Study of behavioral patterns and infection analyses in anopheline species involved in the transmission of malaria in Buriticupu and São José de Ribamar municipality, Maranhão State, Brazil

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Abstract. *Anopheles darlingi* Root and *Anopheles aquasalis* Curry are the main vectors of malaria that occur in the State of Maranhão. Entomological surveys based on the behavior and infectivity of these vectors are important for the elaboration of disease control strategies. The objectives of this work were to study the behavioral patterns of mosquitoes, determining population and hematophagic peaks, dietary preferences, infectivity rate and characterization of breeding sites in two municipalities in the State of Maranhão: Buriticupu and São José of Ribamar. Larvae and pupae were collected in breeding sites and adult females in home environments. Mosquito behavior, their dietary preferences and *Plasmodium* spp. infection rates were analyzed. The vegetation and physicochemical patterns in the breeding sites found are in agreement with those described for species from the Amazon region and the Brazilian Atlantic coast. *Anopheles darlingi* was the most prevalent mosquito in Buriticupu breeding and home environments. This species was found mainly fed on human blood and naturally infected with *Plasmodium vivax* Grassi & Feletti and *Plasmodium falciparum* Welch. *Anopheles aquasalis* was more frequent in breeding sites in São José de Ribamar, as well as in home environments, whose specimens were mainly fed with human and bird blood. The main peaks of mosquito occurrence in Buriticupu were between 6 pm to 9 pm and in São José de Ribamar we did not record a definite peak. In the first municipality *A. darlingi* showed dominance over *Anopheles albitarsis* Lynch Arribálzaga *s.l.*, *Anopheles oswaldoi* Peryassú, *Anopheles nuneztovari* Gabaldón and *Anopheles evansae* Brèthes, besides presenting a correlation with rainfall. In the second municipality, *A. aquasalis* was dominant over *A. albitarsis s.l.* and there was a correlation between these two species and the rainy season. We conclude that the collected data contribute to elucidate the dynamics of malaria transmission in the region and guide the control actions directed to the elimination of the disease in the country.

Keywords: Behavior; Blood Meal; Ecology; Parasite; Vector.

Mosquitoes which belong to *Anopheles* genus have a relevant importance as vectors in public health, due to the potentiality that they must transmit plasmodia parasites that cause the human malaria (Forattini 2002). There were cataloged 57 species of anopheline in Brazil. Among them, at least two were recognized as the main malaria vectors: *Anopheles darlingi* Root, with a vast occurrence in the Amazonian interiors and in Brazil as a whole (Davis 1931; Hiwats & Bretas 2011) and *Anopheles aquasalis* Curry, with a restricted distribution in the coastal strip and adjacencies (Galvão *et al*. 1942; Sinka *et al*. 2010).

The studies of malaria vectors are relevant for the biology knowledge, the behavior of species and the dynamic transmission of plasmodia parasites in each locality, helping in this way to elucidate the disease chain, the prediction of outbreaks and the guidance of control actions (Barbosa *et al*. 2014, 2016). The entomological parameters such as density, anthropophilia, zoophilia, peaks of hematophagic activity, seasonal variations, infection rate and blood-feeding patterns are among the most frequent for the determination of the vector species (Barbosa *et al*. 2014).

In these studies, the relationship between the reduction in density of this group and the Amazonian rainy period was demonstrated, being more evident for the adult forms than the immature forms, since in the rainy season these insects are displaced from permanent breeding sites, from the mainland for lakes and ponds that form along river banks during floods. As the waters rise above the level of the flooded areas they originate the temporary breeding sites located in the middle of the forest. The availability of shelters or breeding sites in the rainy season facilitates the increase of breeding sites and the extension of anopheline occurrence, with a reflection on the prevalence of malaria, as it provides greater contact between man and vector. This is due to the fact that in regions where permanent breeding sites are distanced from human dwellings during the rainy season by the formation of temporary breeding sites, these are close to human dwellings intensifying human contact / vector. The pioneering studies performed by Galvão *et al*. (1942) and Deane *et al*. (1948) concluded that the intense reproduction of *A. darlingi* during the rainy season is associated with the numerous breeding sites in the region during this period, as well as the presence of this vector associated with anthropic action, which promotes changes in the natural habitat of mosquitoes.

These factors condition the emergence of new cases and the onset of epidemics. The process of marked environmental changes, according to Póvoa *et al*. (2003), in the natural environment of the municipality of Belém, conditioned the resurgence of *A. darlingi* from the 90s (twentieth century), after more than twenty years without registration of this species in this area. With environmental impacts, new outbreaks of malaria transmission emerged with possible influence on the cast of local species and changes in the distribution pattern, biology and behavior of these mosquitoes.

The disordered growth of many cities in malaria-endemic areas with inadequate housing conditions and the pressure of human populations, especially the poorest, on the environment, are conditioning factors for malaria maintenance. Vittor *et al.* (2006, 2009) demonstrated in Peru that the pressure of human occupations and activities on the environment positively favors the *A. darlingi* vector that finds new conditions for proliferation, increasing the risks of disease transmission. These conditions were also recorded by Tadei *et al*. (2017) in many Brazilian Amazonian municipalities, which are responsible for the onset of epidemic outbreaks of malaria and their expansion to the major urban centers of the region.

The State of Maranhão, for example, illustrates this scenario well, because besides being considered the poorest in Brazil (IPEA 2019), it has significant areas with environmental degradation where there is active transmission of malaria, both in the coastal range of its territory and in the São José de Ribamar, which accounts for the majority of cases of the disease on the Atlantic coast and Buriticupu in the intracontinental zone. The aid of this paper was study the behavioral patterns of anopheline species in Buriticupu and São José de Ribamar municipalities, in Maranhão state, determining peaks of hematophagic activities, blood-feeding preferences, natural infection rates, breeding sites characterization; the associations among the climate conditions and the presence of the *Anopheles* species; and the associations among the presence of the *Anopheles* species. The data of this work are part of a big project which studies the areas of malaria transmissions in Maranhão, being the first publication of the set or group performed by Barros *et al*. (2015), in which was made the first register for *A. aquasalis* in Buriticupu. Thus, the data that mention the presence of this vector in that municipality was created from the set of this project.

MATERIALS AND METHODS

Study areas

The studies were conducted monthly from January in 2006 to February in 2011, in two localities of Maranhão State in Brazil:

A. Buritizinho village (4º30'34,1" S; 46º49'27,3" W) in municipality of Buriticupu. This locality is intercontinental; 300 km far away from the north Atlantic coast of Brazil and it is located between the borders of Pindaré and Buriticupu rivers, therefore, it is a semi-urban locality The access that place is by BR-222 Highway and by EF-315 dos Carajás Railroad.

B. Guarapiranga village (02º40'52,1" S; 44º08'43,9" W) it is a semi-urban locality in municipality of São José do Ribamar. It is located in the coastal area of the Atlantic Ocean, inside of the Arraial Bay, in São Luís island complex. This area suffers the influence of tidal pulse and of several rivers whose outflow in the bay itself.

Mosquito sampling

Immature mosquitoes. The breeding sites selected for the study were located within 500 meters of human dwellings. In Buriticupu, these breeding sites were mainly characterized as riverbanks and backwaters. In São José de Ribamar, the most common breeding sites were marshes and mangroves.

The immature stages of anopheline were collected in their breeding sites between 7:00 am and 9:00 am through active search using a mosquito larvae dipper. With the obtained data we calculate the Larvae Index per Man Hour – LIMH by the formula:

LIMH= Number of larvae collected / Number of collecting technicians / Number of Collection Hours.

Adult mosquitoes. The adult mosquitoes collections took place intradomicile, peridomicile and in extra-domicile environments, in Buritizinho and in Guarapiranga, in the intradomicile and peridomicile. The peridomicile is the outdoor area surrounding the residence within 200 meters. Nevertheless, the extra domicile corresponds to an area greater than 200 meters radius. The techniques used for catching adult females were by Castro's aspirator: I) human landing catches (HLC), by trained technicians using personal protective equipment, and; II) active search for resting mosquitoes inside houses and in locations where there were animals taking a rest. Both were conducted during a period of time from 6:00 pm to 6:00 am (12 h), stratified or separated by a timetable or schedule; and a time from 6:00 pm to 10:00 pm (4 h). The studies were conducted in six human dwellings in each locality. There were obtained temperature data, relative humidity in the gathering environments, by means of digital thermo-hygrometers, and besides rainfall, in the database of the Instituto Nacional de Meteorologia – INMET.

The collection of mosquito samples conducted in this study is part of the routine work of technicians of the Fundação Nacional de Saúde – FUNASA, authorized by the Instituto Chico Mendes de Conservação da Biodiversidade – ICMBIO (SISBIO Number 18281-1).

Mosquito identification

The collected mosquitoes were taken to the entomology laboratory of the Universidade Federal do Maranhão – UFMA, in São Luís, for identification through the dichotomous keys of Forattini (2002).

Breeding site characterization

Breeding water information such as: temperature, hydrogen potential, electric conductivity, total dissolved solids, salinity, nitrogen compounds, ammonia and phosphorus, as well as the registration of aquatic plants, were also acquired by a descriptive characterization. The identification of vegetable was conducted in laboratories of the Universidade Federal do Maranhão – UFMA, in São Luís.

Blood-feeding preferences

Siqueira's (1960) precipitin technique with modifications was made in order to identify the blood-feeding. Just some female examples of *A. darlingi* and *A. aquasalis* were used in this assay, in which their digestive tubes were removed submitted or kept down in solutions and centrifuged to obtain serum. Right away, they were examined with antisera of birds, mammals, reptiles and amphibians (Lorosa *et al*. 1998). The set of antisera and their respective tested titles were: anti-human 1:15,000; birds 1:10,000; dogs 1:15,000; cats 1:12,000; horses 1:16,000; goats 1:14,000; cattle 1:15,000; porks 1:10,000; sheep 1:8,000; lizards 1:14,000; rodents 1:17,000; possum 1:15,000; armadillos 1:15,000; frogs 1:16,000. All of the Precipitin tests were made in partnership with laboratories of pathology and parasitology of Fundação Oswaldo Cruz - FIOCRUZ, in Rio de Janeiro.

Natural infection by *Plasmodium* spp.

Estimating the rate of infection by *Plasmodium* ssp. in the anopheline female adults samples in both localities, was performed by the Nested-PCR technique, using specific primers according with the description of Snounou *et al*. (1993). Then we calculate the Minimum Infection Rate - MIR by the formula: MRI= ([number of positive pools/total specimens tested]x1,000). These procedures were conducted in the Instituto Nacional de Pesquisas da Amazônia - INPA, in Manaus.

Statistical analyzes

The average of adult mosquitoes collected from January in 2006 to December in 2011, for both environments, were interpreted considering combinations with the average temperature indexes (°C), relative humidity (%) and precipitation (mm). In order to quantify the associations among the climate conditions and the presence of the species and the coexistence of species, we obtained the simple and partial correlation estimates using the software R (R Development Core Team 2013).

RESULTS

Mosquito sampling

In total, 3,110 anopheline specimens were collected from adults and larvae distributed in nine species. They are: *Anopheles albitarsis* s.l. Lynch Arribálzaga, *A. aquasalis*, *A. darlingi*, *Anopheles evansae* Brèthes, *Anopheles galvaoi* Causey, Deane and Deane, *Anopheles nuneztovari* Gabaldón, *Anopheles oswaldoi* Peryassú, *Anopheles strodei* Roots and *Anopheles triannulatus* Neiva e Pinto.

The immature samples in the breeding sites totalized 190 anopheline larvae and five species collected. In Buriticupu, two breeding sites were positive and in São José de Ribamar, only one, out of a total of three, in each inspected locality.

The most frequent species was *A. darlingi* (41.1%) and was found only in Buriticupu, where it represented 78% of the sample. In São José de Ribamar, the most frequent was *A. aquasalis* (61.3%). The total of ILHH was 2.00, being 1.33 in Buriticupu and 0,64 in São José de Ribamar (Table 1).

2.290 females of anophelines were collected, being 2,845 (97%) in Buriticupu and 75 (3%) in São José de Ribamar. In the first locality, among the nine species, *A. darlingi* was the most collected (63.59%) mainly in the peridomicile environment (62.96%). In the second location, *A. aquasalis* was more prevalent (46.67%) among the five species found, being also more frequent in the peridomicile environment (46.48%) (Table 2).

Breeding site characterization

Regarding the water temperature of the breeding sites, very close values were found between, Buriticupu and São José de Ribamar, respectively 21°C and 20°C. The pH varied slightly between the environments, respectively 5.18 and 5.91. The electrical conductivity was 0.263 S/m1 for Buriticupu and 1.24 S/m-1 for São José de Ribamar. The total dissolved solids – TDS 131.00 mg/L in Buriticupu and 23.50 mg/L in São José de Ribamar. The salinity was lower than 0.0001 in Buriticupu and 0.5/1.000 in São José do Ribamar. In the chemical analysis of nitrite in Buriticupu, were found mean values 0.25 mg/L, while in São José de Ribamar, the mean values were 0.75. Regarding nitrate, the Buriticupu samples quantified 1.42 mg/L, while in São José de Ribamar, the values found were higher, 16.15 mg/L. Regarding the ammonia content in the breeding sites, the average value in Buriticupu was 4.63 mg/L. For São José do Ribamar, this value was 21.61 mg/L. The phosphorus content dissolved in Buriticupu breeding sites was on average 57.43 mg/L, and 55.03mg/L in São José do Ribamar (Table 3).

In relation to the presence of associated vegetation, the breeding sites in Buriticupu presented six families and six genera with greater dominance of the genus *Cyperus* sp. In São José de Ribamar, were found five families and five genera with *Avicennia* sp. and *Rizophora* sp. Just the genus *Nymphaea* was present in the breeding sites of both localities. All the evaluated breeding sites were classified as natural, in the soil, permanent or semi-permanent, whose margins are shaded due to the presence of *Mauritia flexuosa*, in Buriticupu and *Avicennia* sp. with *Rizophora* sp. in São José do Ribamar (Table 3).

Blood-feeding preferences

In Buriticupu, a total of 349 females were collected from *A. darlingi* engorged, 64 intradomicile, 139 in peridomicile, and 146 in extradomicile. About *A. aquasalis* 17 specimens were collected, 4 intradomicile and 13 in peridomicile. In intradomicile, 7.73% of *A. darlingi* collected, were fed human blood, followed by 5.17% with blood from birds and 2.86% with blood from cattle. In peridomicile, the highest number of *A. darlingi* fed with human blood (14.32), followed by dog blood (10.02%) and the third highest value for bovine blood (6.59%). The extradomicile data differ from the pattern observed in both environments, with the highest value for bird blood (13.75%), followed by human blood (12.32%) and cattle blood the third most frequent (6.59%). Considering the general total, the *A. darlingi* by human blood represented 34.38% of the samples, followed by bird – 23.49% and cattle the third most frequent, representing 16.04% of the total. Whereas *A. aquasalis,* the percentage of specimens was very low, of which 13 specimens were found in peridomicile, fed mostly with human blood (58.82%) and another 4 in intradomicile fed with human blood (11.76%) and bird (11.76%) (Table 4).

The analysis of the precipitin double reaction for blood-feeding of *A. darlingi* and *A. aquasalis* allowed detecting that in intradomicile, 21 specimens (14.38%) of *A. darlingi* were fed on rodent / human blood and 17 (11.64%) on bird / human blood. Only one female from *A. aquasalis* fed on bird / human. In peridomicile, 19 specimens of *A. darlingi* (13.01%) fed on bird / human blood, while 14 (9.58%) fed on cat / bird and 6 (4.10%) on poultry / cattle blood. In extradomicile, 20 specimens from *A. darlingi* (13.69%) were engorged with human / skunk blood. There were also 18 specimens (12.32%) of the skunk / bird combination; 16 (10.95%) bird / cattle and 15 (10.27%) of the bird / dog combination. Considering the general total of the double reactions, the *A. darlingi* per bird / human, represented 24.65% of the mosquitoes collected, followed by the skunk / bird combination (21.91%) and 15.06% for the bird / cattle combination. For *A. aquasalis* reacted doubly only one specimen for the bird / human (Table 4).

Table 1. Densities of *Anopheles* spp. larvae collected in breeding sites in the municipalities of Buriticupu and São José de Ribamar, Maranhão, State, from 2006 to 2011.

Species	Buriticupu		São José de Ribamar		Total/Species	
	n	%	n	%	n	%
Anopheles albitarsis	32	25.0	14	22.6	46	24.2
Anopheles aquasalis	0	0,0	38	61.3	38	20.0
Anopheles darlingi	78	60.9	0	0.0	78	41.1
Anopheles nuneztovari	12	9.4	6	9.7	18	9.5
Anopheles oswaldoi	6	4.7	4	6.5	10	5.3
Total/locality	**128**	**-**	**62**	**-**	**190**	**-**
LIMH	**1.33**	**-**	**0.64**	**-**	**2.00**	**-**

LIMH= larvae index per man/hour.

Table 2. Densities of adult *Anopheles* spp. collected in three breeding sites of Buriticupu and São José de Ribamar municipalities, Maranhão State, from 2006 to 2011.

Species	Environment						Total/Species	
	Intradomicile		Peridomicile		Extradomicile			
	n	%	n	%	n	%	n	%
Buriticupu								
Anopheles albitarsis	156	26.17	348	21.27	149	24.31	653	22.95
Anopheles aquasalis	4	0.67	13	0.79	0	0.00	17	0.60
Anopheles darlingi	389	65.27	1,030	62.96	390	63.62	1,809	63.59
Anopheles evansae	0	0.00	92	5.62	0	0.00	92	3.23
Anopheles galvaoi	3	0.50	5	0.31	10	1.63	18	0.63
Anopheles nuneztovari	5	0.84	41	2.51	26	4.24	72	2.53
Anopheles oswaldoi	39	6.54	85	5.20	37	6.04	161	5.66
Anopheles strodei	0	0.00	12	0.73	1	0.16	13	0.46
Anopheles triannulatus	0	0.00	10	0.61	0	0.00	10	0.35
Total/environment	**596**	**-**	**1,636**	**-**	**613**	**-**	**2,845**	**-**
São José de Ribamar								
Anopheles albitarsis	1	25.00	22	30.99	ns	-	23	30.67
Anopheles aquasalis	2	50.00	33	46.48	ns	-	35	46.67
Anopheles darlingi	1	25.00	4	5.63	ns	-	5	6.67
Anopheles oswaldoi	0	0.00	9	12.68	ns	-	9	12.00
Anopheles nuneztovari	0	0.00	3	4.23	ns	-	3	4.00
Total/environment	**4**	**-**	**71**	**-**	**-**	**-**	**75**	**-**
Total							**2,920**	

ns = non-sampled environment

In São José de Ribamar, the precipitin tests revealed that 20 females (80.00%) of *A. aquasalis* fed on human blood, being two intradomicile (8.00%) and 18 (72%) in peridomicile. The tests also revealed that five (20%) females caught in peridomicile had fed on birds. Considering the results of the double precipitin reaction, only three (20.00%) of the female caught in peridomicile revealed the combination of cat / human feeding and 12 specimens (80.00%) also in peridomicile the human / bird combination (Table 4).

Correlation between *Anopheles* species

In Buriticupu, we detected that *A. darlingi* coexists positively in significance with *A. albitarsis s.l., A. oswaldoi, A. nuneztovari* and *A. evansae* in Buriticupu. However, *A. albitarsis s.l.* was dominant on the same species, with the exception of *A. darlingi.* The behavior of these species reveals the potential of *A. darlingi* as the main vector in the environment and *A. albitarsis s.l.* as an associated vector. In São José de Ribamar, the coexistence of the species was significant for *A. aquasalis* and *A. albitarsis s.l.*, and between *A. oswaldoi* and *A. nuneztovari.* The species *A. darlingi* and *A. albitarsis s.l.* coexist in that locality in a positive way among the evaluated environments, in the same way as *A. oswaldoi* and *A. nuneztovari* (Table 5).

Correlation between *Anopheles* species and climate variables

Of all the climatic data analyzed, only rainfall showed a significant correlation with *A. darlingi* in Buriticupu and with *A. albitarsis s.l.* and *A. aquasalis* in São José de Ribamar (Table 6). During the period of this study, in both localities, the monthly variations of temperature and humidity had few differences in amplitudes. However, the rainfall regime was decisive for the maintenance of *A. darlingi* in Buriticupu (P = 0.0280) (Figure 1) and for *A. aquasalis* (P= 0.0051) (Figure 2) in São José de Ribamar. These data indicate that the presence of these species was strongly related to the rainy seasons in both localities.

Peak of hematophagy

The biting activity of the females in Buriticupu, behaved in a very similar way for the species *A. darlingi, A. albitarsis s.l., A. nuneztovari* and *A. oswaldoi* whose peaks occurred in the first two schedules in practically all of the evaluated environments. *Anopheles darlingi* reached 7.0 mosquito/man/hour index in intradomicile between 6:00 and 7:00 pm and 10.9 between 7:00 and 8:00 pm. In peridomicile at the same time, the rates of mosquito/man/hour were 14.4 and 42.1, respectively. Already in extradomicile, were 6.9 and 10.9 mosquito/man/hour. For *A. albitarsis s.l.* the highest indexes were reached in peridomicile between 6:00 and 7:00 pm and from 7:00 to 8:00 pm respectively, 7.0 and 10.0 mosquito/man/hour. In the species *A. nuneztovari* and *A. oswaldoi* the indexes were lower than one (Figure 3). In São José de Ribamar, only mosquitoes were collected during 12-hour samplings in a peridomicile environment. The mosquito/man/hour indexes were considered very low, varying between 0,00 and 0,07, in this case it wasn't possible to detect a well-defined period of the species' peak (Figure 4).

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Table 4. Results of the simple and double reaction of the precipitin for the analysis of the *Anopheles darlingi* and *Anopheles aquasalis* blood-feeding captured intradomicile, peridomicile and extradomicile environments in Buriticupu and São José de Ribamar municipalities, Maranhão State, from 2006 to 2011.

Feeding source	Intradomicile		Peridomicile		Extradomicile		Total	
	Anopheles darlingi n (%)	*Anopheles aquasalis* n (%)	*Anopheles darlingi* n (%)	*Anopheles aquasalis* n (%)	*Anopheles darlingi* n (%)	*Anopheles aquasalis* n (%)	*Anopheles darlingi* n (%)	*Anopheles aquasalis* n (%)
Buriticupu: simple reaction								
Human	27 (7.73)	2 (11.76)	50 (14.32)	10 (58.82)	43 (12.32)	0	120 (34.38)	12 (70.58)
Cattle	10 (2.86)	0	23 (6.59)	2 (11.76)	23 (6.59)	0	56 (16.04)	2 (11.76)
Bird	18 (5.15)	2 (11.76)	16 (4.58)	0	48 (13.75)	0	82 (23.49)	2 (11.76)
Rodent	6 (1.71)	0	13 (3.72)	0	11 (3.15)	0	30 (8.59)	0
Skunk	0	0	0	0	12 (3.43)	0	12 (3.43)	0
Dog	0	0	35 (10.02)	0	5 (1.43)	0	40 (11.46)	0
Cat	0	0	0	1 (5.88)	0	0	0	1 (5.88)
Not reacted	3 (0.85)	0	2 (0.57)	0	4 (1.14)	0	9 (2.57)	0
Subtotal	**64 (18.33)**	**4 (23.52)**	**139 (39.82)**	**13 (76.47)**	**146 (41.83)**	**0**	**349**	**17**
Buriticupu: double reaction								
Bird/Human	17 (11.64)	1 (100.00)	19 (13.01)	0	0	0	36 (24.65)	1 (100.00)
Rodent/ Human	21 (14.38)	0	0	0	0	0	21 (14.38)	0
Human/Skunk	0	0	0	0	20 (13.69)	0	20 (13.69)	0
Bird/Dog	0	0	0	0	15 (10.27)	0	15 (10.27)	0
Skunk/Bird	0	0	14 (9.58)	0	18 (12.32)	0	32 (21.91)	0
Bird/Cattle	0	0	6 (4.10)	0	16 (10.95)	0	22 (15,06)	0
Subtotal	**38 (26.02)**	**1 (100.00)**	**39 (26.71)**	**0**	**69 (47.26)**	**0**	**146**	**1**
São José de Ribamar: simple reaction								
Human	-	2 (8.00)	-	18 (72.00)	-	-	-	20 (80.00)
Bird	-	0	-	5 (20.00)	-	-	-	5 (20.00)
Subtotal	**-**	**2 (8.00)**	**-**	**23 (92.00)**	**-**	**-**	**-**	**25**
São José de Ribamar: double reaction								
Cat/Human	-	-	-	3 (20.00)	-	-	-	3 (20.00)
Human/Bird	-	-	-	12 (80.00)	-	-	-	12 (80.00)
Subtotal	**-**	**-**	**-**	**15 (100.00)**	**-**	**-**	**-**	**15**

Natural infection by *Plasmodium* spp.

The rates of natural infection could only be analyzed in Buriticupu, due to the more expressive volume of specimens captured. Only one pool was positive *Plasmodium vivax* and four for *Plasmodium falciparum* in *Anopheles darlingi,* whose MIR were respectively 0,6% and 2.2%. *Anopheles albitarsis s.l.* positivized a pool for *P. vivax* and MIR of 1.1%. *Anopheles nuneztovari* positivized for *P. vivax* and one for *P. falciparum,* whose MIR were 8.3% and 1.1 %. *Anopheles oswaldoi* was positive for *P. falciparum* and MIR 2.3% and also presented positivity for mixed infection, MIR 2.3% (Table 7). The analysis of the distribution of *Anopheles* spp. naturally infected by *Plasmodium* spp. guide us to the conclusion that *A. darlingi* was found infected in the three environments, ranging in the first four hours of the evening and closer to dawn. *Anopheles albitarsis s.l.* infected occurred in peridomicile and in extradomicile during the first two hours of the evening, respectively. *Anopheles oswaldoi* was found infected in intradomicile and peridomicile also, during the first two nocturnal times. *Anopheles nuneztovari* was found infected in the peridomicile between 10:00 and 11:00 pm (Figure 5).

DISCUSSION

The records of *Anopheles* species found in this study, were reported for the first time in Estado do Maranhão in Deane *et al*. (1948) which accomplished *Anopheles* sp. fauna collects in different locations, from the Amazon region to the coastal strip of the Brazilian northeast, from 1939 to 1944. The data that we collected enabled to register the predominance of the species *A. aquasalis, A. darlingi* and *A. albitarsis s.l.,* mainly on the island of São Luís, denoting a pattern of occurrence similar to that one found in this work.

The breeding sites of anophelines in Buriticupu and in São José de Ribamar present shading characteristics and a presence of aquatic plants similar to those described by Consoli & Lourenço-de-Oliveira (1994), Moutinho *et al*. (2011) and Forattini (2002) for the immature forms of the species recorded here. It should be noted that these breeding sites are located very close to the peridomicile, being this a conditioning factor for the continuous presence of these vectors during the whole year (Moutinho *et al*. 2011). The type of influence that the presence of aquatic plants along with the limnological conditions in the different kinds of breeding sites, exert on the survival and density of the larvae has been well discussed, since the species tolerate extreme ranges of variations in the biotic and abiotic conditions of these environments (Pinault & Hunter 2012; Soleimani-Ahmadi *et al*. 2014). Such conditions are essential for species commonly found in freshwater (Savage *et al*. 1990) as in the case of *A. darlingi, A. albitarsis s.l., A. nuneztovari* and *A. oswaldoi;* as for those which are tolerant to certain levels of salinity (Savage *et al*. 1990) as *A. aquasalis.* The breeding sites of *A. aquasalis,* in particular, are influenced

Table 5. Estimates of partial correlations between adult *Anopheles* spp. (coexistence) in Buriticupu and in São José de Ribamar municipalities, Maranhão State, from 2006 to 2011.

Location	Pairs of variables	r Simple	r Partial	Correlation	Likelihood
Buriticupu	***Anopheles darlingi* x *Anopheles albitarsis***	**0,72**	**0,776**	**9.6873****	**0,00001**
	Anopheles darlingi* x *Anopheles oswaldoi	**0,17**	**0,295**	**2.4314***	**1,7134**
	Anopheles darlingi x *Anopheles strodei*	0	-0,0279	-0.2201ns	82,131
	Anopheles darlingi* x *Anopheles nuneztovari	**0,21**	**0,3746**	**3.1814****	**0,2417**
	Anopheles darlingi x *Anopheles galvaoi*	0,07	0,0998	0.7898ns	56,1646
	Anopheles darlingi* x *Anopheles evansae	**0,21**	**0,362**	**3.0576****	**0,3388**
	Anopheles albitarsis* x *Anopheles oswaldoi	**-0,02**	**-0,2564**	**-2.0889***	**3,8655**
	Anopheles albitarsis x *Anopheles strodei*	0,02	0,0388	0.3057ns	75,8651
	Anopheles albitarsis* x *Anopheles nuneztovari	**-0,04**	**-0,3264**	**-2.7193****	**0,8291**
	Anopheles albitarsis x *Anopheles galvaoi*	-0,03	-0,1181	-0.9365ns	64,4759
	Anopheles albitarsis* x *Anopheles evansae	**0**	**-0,2684**	**-2.1938***	**3,0314**
	Anopheles oswaldoi x *Anopheles strodei*	-0,08	-0,023	-0.1815ns	85,0987
	Anopheles oswaldoi x *Anopheles nuneztovari*	-0,01	-0,0967	-0.765ns	54,6767
	Anopheles oswaldoi x *Anopheles galvaoi*	0,07	0,0172	0.1357ns	88,7787
	Anopheles oswaldoi x *Anopheles evansae*	-0,07	-0,1797	-1.4382ns	15,1545
	Anopheles strodei x *Anopheles nuneztovari*	-0,02	-0,0351	-0.2762ns	77,9814
	Anopheles strodei x *Anopheles galvaoi*	-0,09	-0,0937	-0.7413ns	53,2293
	Anopheles strodei x *Anopheles evansae*	0,14	0,1341	1.0657ns	29,0937
	Anopheles nuneztovari x *Anopheles galvaoi*	-0,07	-0,1215	-0.9641ns	65,9329
	Anopheles nuneztovari x *Anopheles evansae*	0,01	-0,0696	-0.549ns	59,1715
	Anopheles galvaoi x *Anopheles evansae*	0,03	0,0134	0.1057ns	91,2711
São José de Ribamar	***Anopheles aquasalis* x *Anopheles albitarsis***	**0,94**	**0,9153**	**18.1843****	**0,00001**
	Anopheles darlingi x *Anopheles aquasalis*	0,37	-0,0003	-0.0024ns	99,3478
	Anopheles darlingi x *Anopheles oswaldoi*	-0,04	0,0404	0.3237ns	74,5862
	Anopheles darlingi x *Anopheles nuneztovari*	-0,02	-0,0625	-0.5013ns	62,3665
	Anopheles albitarsis x *Anopheles aquasalis*	0,42	0,0463	0.3709ns	71,2857
	Anopheles albitarsis x *Anopheles oswaldoi*	-0,05	-0,0495	-0.3968ns	69,4932
	Anopheles albitarsis x *Anopheles nuneztovari*	0	0,0587	0.4703ns	64,4675
	Anopheles aquasalis x *Anopheles oswaldoi*	-0,1	-0,0515	-0.4124ns	68,4191
	Anopheles aquasalis x *Anopheles nuneztovari*	-0,03	-0,041	-0.3283ns	74,2676
	Anopheles oswaldoi* x *Anopheles nuneztovari	**0,28**	**0,2929**	**2.4509***	**1,6242**

*= significant result; ns= no significant.

by the cycles of tides and rivers that flow into the estuary of Arraial Bay, near Guarapiranga. As reported by Moser *et al*. (2004) the immature habitats of this species correlated positively with the alkalinity and salinity of the water, typically of mangroves.

The physico-chemical characteristics of the breeding sites in both localities are similar to the data obtained by Savage *et al*. (1990) in Chiapas, Mexico and with Tadei *et al*. (1993) in Amazonas, Brazil. The value of TDS[2] found, was lower than the findings of Claborn *et al*. (2002) in South Korea. As for the nitrite, nitrate, ammonia and phosphorus values, the records of Mwangangi *et al*. (2007) report that *Anopheles arabiensis* Patton in flooded rice plantations, in Kenya, are tolerant to different levels of this chemical compound. The LIMHs were compatible with those obtained in the controls of Rodrigues *et al*. (2008) and lower than the controls of Ferreira *et al*. (2015) in artificial breeding sites, such as potholes and pisciculture tanks, respectively.

The results enabled to verify that the anophelines fauna among home environments was more frequent in peridomicile. In this environment are located people at dusk and in the early hours of the night, as well as domestic animals and some occasional wild ones. In this context, it becomes possible to know the anophelines fauna, allowing to assess the level of risk of malaria transmission. Considering the densities of the vectors, the data found in Buriticupu, were more expressive than those obtained in the following municipalities: Paço do Lumiar (199 specimens), São Luiz (2,132 specimens), Raposa (1,215) and São José de Ribamar (229 specimens) by Rebêlo *et al*. (2007). The most plausible explanation for the presence of these species is the geographic position of Maranhão, which is located in the semi-humid transition zone between hot and humid climate with predominance of ombrophilous forest and semi-arid with drier vegetation. However, in São José de Ribamar, the register of the species was low, 75 in total, when comparing the works of Rebêlo *et al*. (2007) carried out in the same locality. Nevertheless, our data corroborate those obtained in the state, both in Buriticupu and in the island of São Luis (Rebêlo *et al*. 2007; Silva *et al*. 2006).

Anopheles darlingi presented a positive correlation with four vectors *A. albitarsis s.l., A. oswaldoi, A. nuneztovari* and *A. evansae* and from *A. albitarsis s.l.* with a positive correlation with *A. evanse* and negative with *A. nuneztovari* and *A. oswaldoi* in Buriticupu. Rebêlo *et al*. (2007) reported the coexistences in the municipality, especially in periods of malaria transmissions. Positive correlations between *A. aquasalis* and *A. albitarsis s.l.* and *A. oswaldoi* and *A. nuneztovari* were also obtained; these findings are corroborated by Silva *et al*. (2006) and Rebêlo *et al*. (2007) for São José de Ribamar. The rainy season was a condition for the maintenance of *A. darlingi, A. albitarsis s.l.* and *A. aquasalis* in both locations. Galardo *et al*. (2009) found a significant correlation between *A. darlingi* and the rainy season. Other reports have shown relevant densities for these vectors in Amazonian regions wettest periods (Tadei *et al*. 1998; Moutinho *et al*. 2011; Barbosa *et al*. 2014) and the island of São Luiz (Xavier & Rebêlo 1999). In the rainy season, the availability of natural breeding sites increases, providing new breeding sites for anophelines, a hegemonic factor for expanding the densities of these mosquitoes.

Anopheles darlingi was the species that presented the greatest endophilic behavior, revealing the ability that this mosquito has to make the blood-feeding or hematophagy on humans

Table 6. Estimates of partial correlations between adult *Anopheles* species and climate variables in Buriticupu and in São José de Ribamar municipalities, Maranhão State, from 2006 to 2011.

Location	Pairs of variables	r Simple	r Partial	Correlation	Likelihood
Buriticupu	Temperature x *Anopheles darlingi*	-0,06	0,0001	0.0008ns	99,4708
	Temperature x *Anopheles albitarsis*	-0,09	-0,0479	-0.3778ns	70,8115
	Temperature x *Anopheles oswaldoi*	-0,04	-0,0491	-0.3867ns	70,1942
	Temperature x *Anopheles strodei*	0,14	0,1494	1.1897ns	23,6966
	Temperature x *Anopheles nuneztovari*	-0,1	-0,0334	-0.2634ns	78,9111
	Temperature x *Anopheles galvaoi*	0,01	0,0358	0.2818ns	77,5759
	Temperature x *Anopheles evansae*	0,27	0,1753	1.4023ns	16,2118
	Rain x *Anopheles darlingi*	**0,34**	**0,2723**	**2.2282***	**2,7958**
	Rain x *Anopheles albitarsis*	0,19	-0,088	-0.6955ns	50,37
	Rain x *Anopheles oswaldoi*	0,17	0,0713	0.5629ns	58,2459
	Rain x *Anopheles strodei*	-0,07	-0,0437	-0.3443ns	73,1444
	Rain x *Anopheles nuneztovari*	-0,02	-0,1015	-0.8033ns	56,9712
	Rain x *Anopheles galvaoi*	0,16	0,119	0.9434ns	64,8407
	Rain x *Anopheles evansae*	0,04	-0,0511	-0.4031ns	69,0654
	Relative humidity x *Anopheles darlingi*	0,1	0,1088	0.8616ns	60,3463
	Relative humidity x *Anopheles albitarsis*	0,07	-0,05	-0.3943ns	69,6746
	Relative humidity x *Anopheles oswaldoi*	-0,09	-0,1395	-1.1089ns	27,1245
	Relative humidity x *Anopheles strodei*	0,08	0,1623	1.2951ns	19,7148
	Relative humidity x *Anopheles nuneztovari*	0,21	0,1512	1.2047ns	23,0956
	Relative humidity x *Anopheles galvaoi*	0,05	0,0924	0.731ns	52,5915
	Relative humidity x *Anopheles evansae*	-0,21	-0,1947	-1.5628ns	11,91
São José de Ribamar	Temperature x *Anopheles darlingi*	-0,1	0,0165	0.1318ns	89,0997
	Temperature x *Anopheles albitarsis*	-0,1	-0,0707	-0.567ns	57,9678
	Temperature x *Anopheles aquasalis*	-0,11	-0,1713	-1.3907ns	16,5481
	Temperature x *Anopheles oswaldoi*	0,01	0,0018	0.0144ns	98,5492
	Temperature x *Anopheles nuneztovari*	0,08	0,0481	0.3854ns	70,2813
	Rain x *Anopheles darlingi*	0,55	-0,2206	-1.8094ns	7,1587
	Rain x *Anopheles albitarsis*	**0,65**	**0,4257**	**3.7632****	**0,0456**
	Rain x *Anopheles aquasalis*	**0,51**	**0,3409**	**2.9005****	**0,5117**
	Rain x *Anopheles oswaldoi*	-0,03	0,035	0.2805ns	77,6724
	Rain x *Anopheles nuneztovari*	0,03	0,0165	0.1316ns	89,1131
	Relative humidity x *Anopheles darlingi*	-0,05	-0,0173	-0.1387ns	88,5285
	Relative humidity x *Anopheles albitarsis*	-0,06	0,0287	0.2299ns	81,3889
	Relative humidity x *Anopheles aquasalis*	-0,23	-0,2221	-1.8227ns	6,9585
	Relative humidity x *Anopheles oswaldoi*	0,13	0,1442	1.1662ns	24,6475
	Relative humidity x *Anopheles nuneztovari*	-0,09	-0,1232	-0.9936ns	67,4717

*= significant result; ns= no significant.

Table 7. Minimum infection rate for natural infections of *Plasmodium* ssp. in four species of *Anopheles* captured in Buriticupu, Maranhão, State, 2006 a 2011.

Species	n	Pools / species	*Plasmodium vivax*		*Plasmodium falciparum*		*P. vivax + P. falciparum*	
			Positive Pools	MIR %	Positive Pools	MIR %	Positive Pools	MIR %
Anopheles albitarsis	238	90	1	1.1	1	1.1	0	0
Anopheles darlingi	804	178	1	0.6	4	2.2	0	0
Anopheles nuneztovari	19	12	1	8.3	1	1.1	0	0
Anopheles oswaldoi	238	44	0	0	1	2.3	1	2.3
Total	**1,299**	**324**	**3**	**-**	**7**	**-**	**1**	**-**

MIR = Minimum infection rate.

inside the houses. In addition, it was also the most frequent in peridomicile and extradomicile. The first scottophase schedules were the ones with the highest mosquito/man/hour indexes with a strong trend of bimodal behavior in Buriticupu. These behaviors were similar to those observed in works carried out in other locations in the Amazon (Deane 1986; lourenço-de-oliveira *et al*. 1989; Tadei *et al*. 1993; Tadei *et al*. 2000; Tadei *et al*. 2017). Nevertheless, the biting activity of *A. aquasalis* during nighttime intervals was also reported in peridomicile (Xavier & Rebêlo 1999). In general, mosquito/man/hour indexes of the vectors differ greatly in each region, at certain times and between species (Lourenço-de-Oliveira *et al*. 1989; Galardo *et al*. 2009; Póvoa *et al*. 2009; Barbosa *et al*. 2016). Whereas the peridomicile was the most affluent and abundant species occurrence environment, allied to human habits in the main peak times of mosquitoes, this information points to guidance of differentiated control measures in this mean, since it has an important role in malaria transmission (Vezenegho *et al*. 2016).

It should be noted that in São José de Ribamar, five specimens of *A. darlingi* were found. The record of *A. darlingi* in the island of São Luís had been made by Deane *et al*. (1948) in which they stated: "*In an experiment done in 1941, freshly hatched larvae of A. darlingi captured in São Luiz (Maranhão)*" [...]. The data of this work corroborate this affirmation because since that publication, no record of the species had been done in that island. Another important fact was the first record of *A. aquasalis* in Buriticupu; 300 km distant from the Atlantic coast (Barros *et al*. 2015).

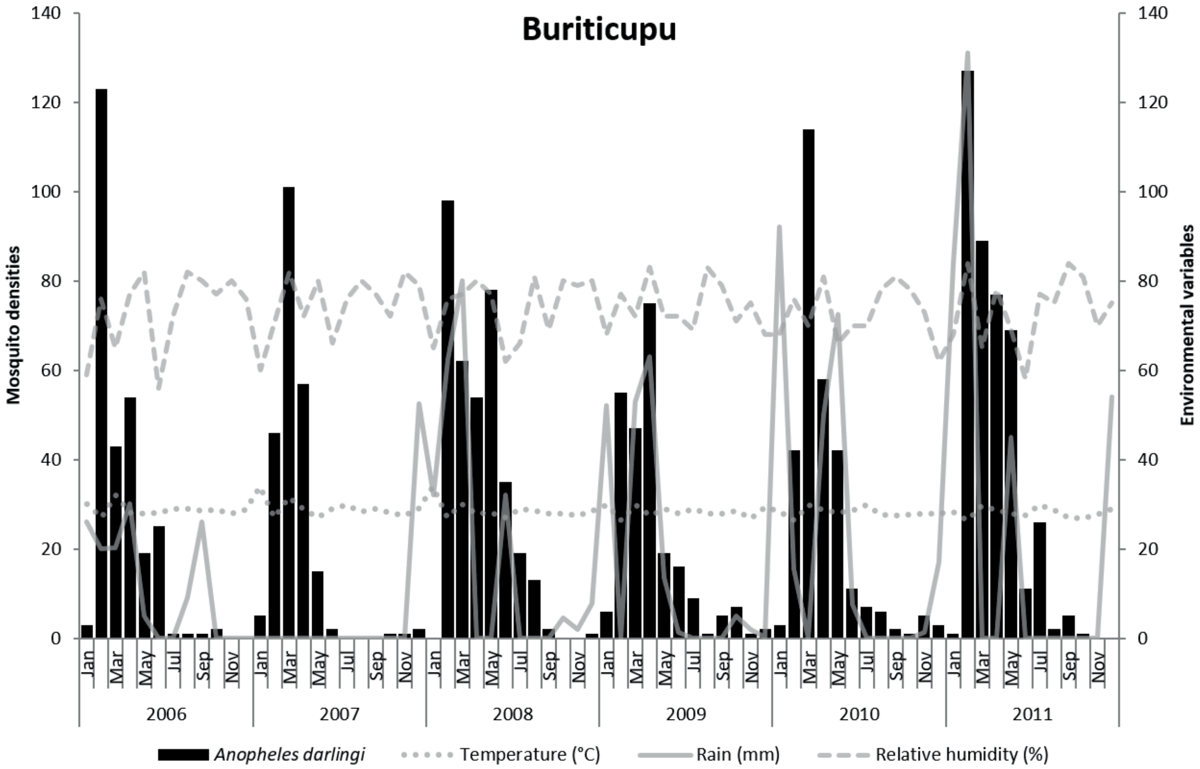

Figure 1. Densities of *Anopheles darlingi* mosquitoes and temperature averages, rainfall and relative humidity in Buriticupu, Maranhão State, from 2006 to 2011.

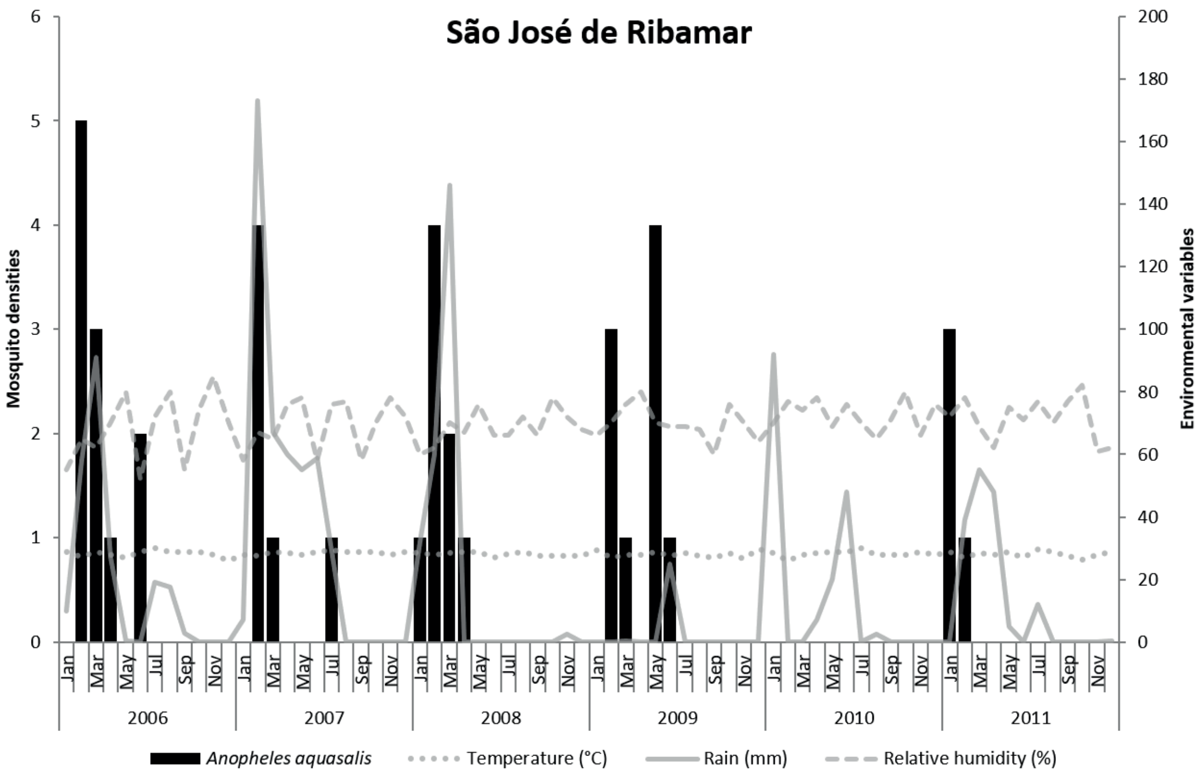

Figure 2. Densities of *Anopheles aquasalis* mosquitoes and temperature averages, rainfall and relative humidity in São José de Ribamar, Maranhão State, from 2006 to 2011.

The blood feeding of the two main vectors, *A. darlingi* and *A. aquasalis* allow to infer that they are very eclectic species feeding on several hosts. However, the higher frequencies of the reactions revealed that both presented a remarkable anthropophilic behavior, as observed in the experiments conducted by Deane *et al*. (1949). In São José de Ribamar, samplings proved the preference of *A. aquasalis* to feed on humans (80%). At this location the other animals hosts were birds and cats. This data differs from what was found by Flores-Mendoza *et al*. (1996), in Rio de Janeiro, who emphasized that this species exhibits zoophilic behavior. However, Deane (1986), points out that the vector capacity of this species is related to the density of the population, whose effects are more pronounced in the north and northeast coast of Brazil.

The natural infection detected four species infected with *Plasmodium* ssp. in Buriticupu – *A. darlingi, A. albitarsis s.l., A. oswaldoi* and *A. nuneztovari.* Notoriously, *A. darlingi,* stands out being infected with both *P. vivax* and *P. falciparum* in the three environments analyzed, reason why it is considered the

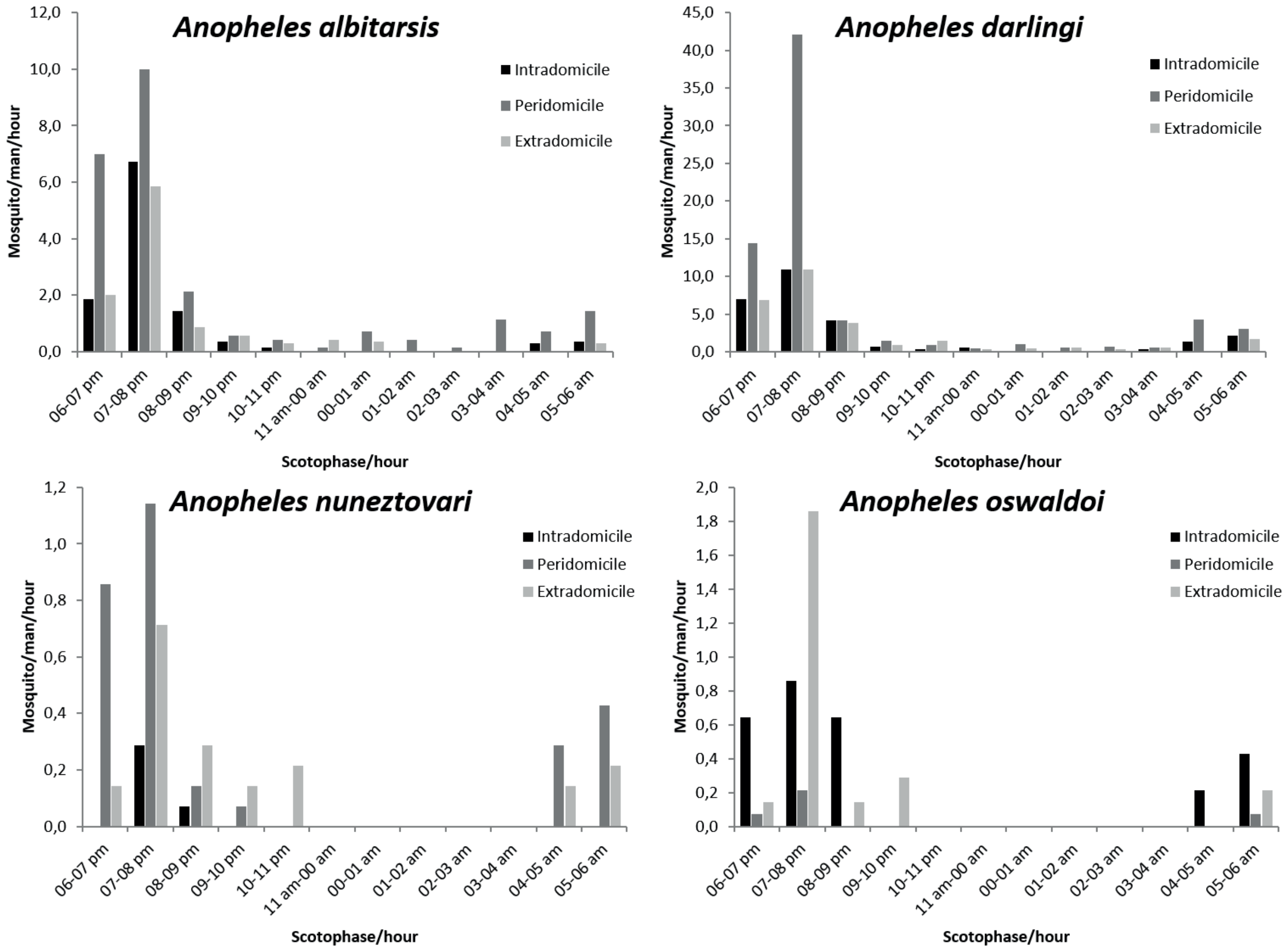

Figure 3. Mosquito / man / hour indexes for *Anopheles* spp. collected in three enviroments and activity schedules in Buriticupu, Maranhão State, from 2006 to 2011.

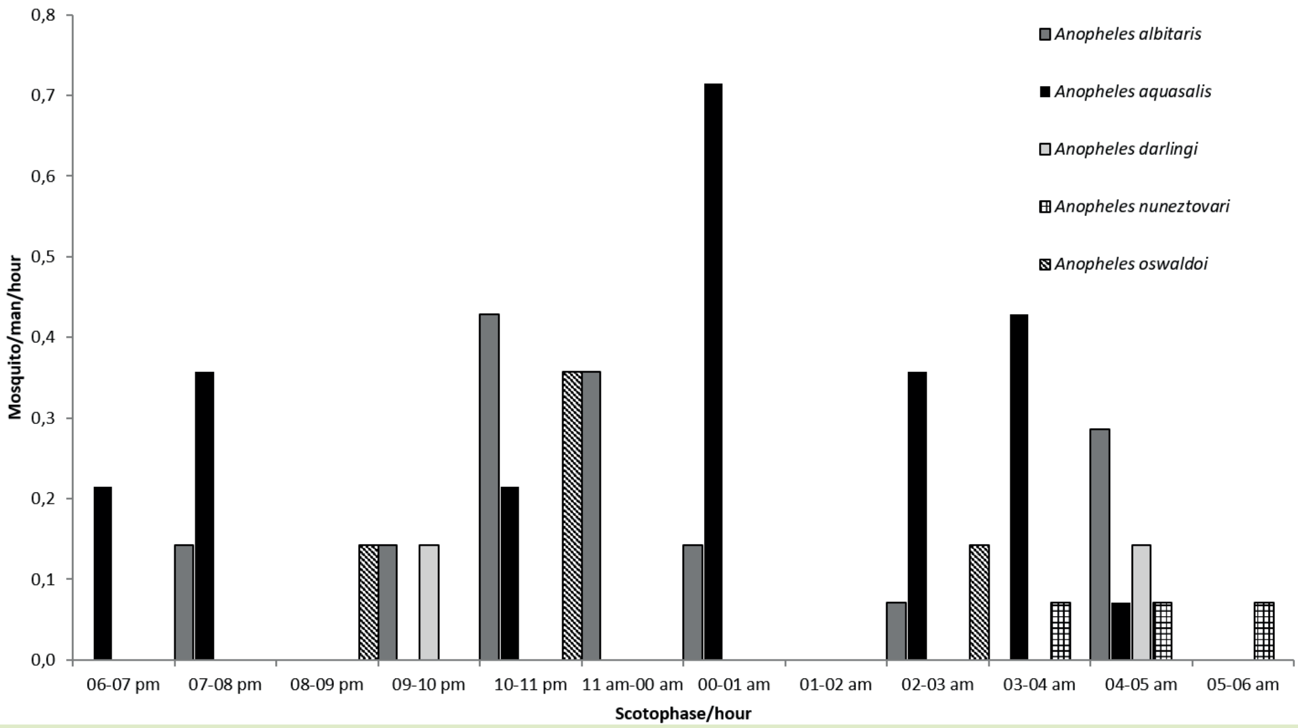

Figure 4. Mosquito / man / hour indexes for species of *Anopheles* collected in a peridomicile environment and activity schedules in São José de Ribamar, Maranhão State, from 2006 to 2011.

main vector of malaria in the region (Tadei *et al*. 1988; Tadei & Dutary-Thatcher 2000; Hiwat & Bretas 2011; Pimenta *et al*. 2015). Although infection rates have been low, this doesn't mean that the risk of transmission in the area is lower, since low parasitemias can trigger successive cycles of epidemics (Klein *et al*. 1991; Alves *et al*. 2005).

In the present study, the three main vectors of malaria in Brazil were detected in the evaluated areas: *A. darlingi, A. aquasalis* and *A. albitarsis s.l.,* whose densities justify their dominance in the environment. Secondary vectors, such as, *A. nuneztovari* and *A. oswaldoi* were also found, however, in a smallest proportion. Nevertheless, as a whole, except for

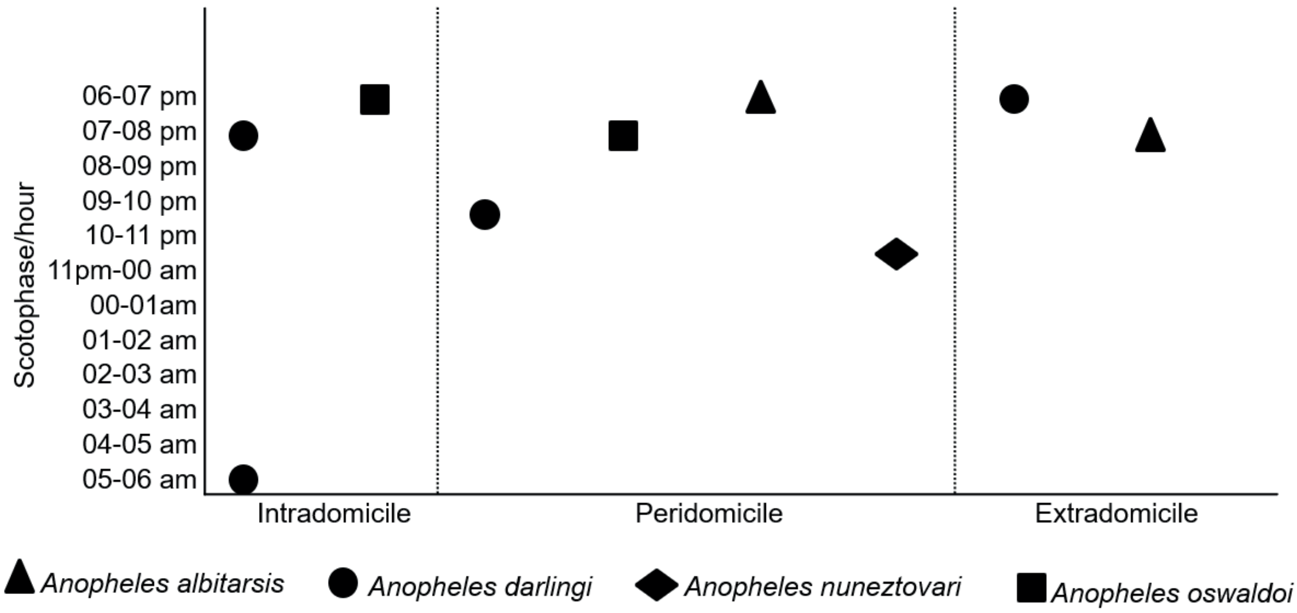

Figure 4. Distribution of the natural infectivity of *Plasmodium* ssp. in *Anopheles* ssp. by schedule and environment in Buriticupu municipality, Maranhão State, from 2006 to 2011.

A. aquasalis, all of them had natural infections by *Plasmodium* ssp. Therefore, have a relevant role in the transmission of malaria, in the municipalities of Maranhão. The volume of information collected allows us to state that *Anopheles darlingi* remains as the main vector of malaria in Buriticupu. As *A. aquasalis* probably has important highlight in the transmission of the disease in São José de Ribamar.

Thus, both locations a high risk for malaria transmission. Finally, the data collected by this work contribute to elucidate a part of the transmission chain in both localities, as well as, serve to guide the control actions by the public health service and the population in general, so that they can adopt conduits to prevent new infections.

In addition, these data are important because they also contribute to the process of controlling and eliminating malaria, an activity which is relevant today, in the Amazon region. The areas of occurrence records of the species of *Anopheles* are the references for the implementation of specific actions, whose ultimate goal is the elimination of this disease, initially focusing on the *P. falciparum* (Santelli *et al.* 2016).

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