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



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### ABSTRACT

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

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### Introduction

Malaria is one of the most epidemiologically important parasitic diseases in the world, due to the high number of cases and deaths, mainly affecting the African continent (WHO, 2016; Stevenson and Norris, 2017). In Brazil, states located in the Amazon region exhibit a higher incidence of this disease compared to other areas, totaling approximately 99% of the registered cases, with some reports from other regions (Brazil, 2017). Certain factors contribute to the maintenance of this vector, and, consequently, malaria, in the Amazon, such as the presence of a tropical forest, which shows favorable geographical and ecological characteristics for the interaction of the

*Plasmodium* parasite and *Anopheles* mosquitoes, socio-cultural population conditions and migratory movements (Tadei et al., 1998; Martens and Hall, 2000).

In Brazil, the subgenus *Nyssorhynchus* is responsible for perpetuating malaria in the Amazon region, while *Kerteszia* is responsible for transmission in areas with Atlantic Rainforest coverage (Branquinho et al., 1997; Forattini, 2002; Rebêlo et al., 2007). The main malaria transmitter in the Amazon region is *An. (Nys.) darlingi* Root, 1926 (Tadei et al., 2017) and other species, such as *An. (Nys.) albitarsis* s.l. Arribáizaga, 1878 (Consoli and Lourenço-de-Oliveira, 1994), *An. (Nys.) nuneztovari* Gabaldon, 1940, *An. (Nys.) triannulatus* Neiva and Pinto, 1922, *An. (Nys.) braziliensis* Chagas, 1907 (Tadei and Dutary-Thatcher, 2000), are considered occasional or secondary vectors.

Mosquitoes use natural and artificial water bodies, such as ponds, riverbanks, puddles and soaked fields, for oviposition

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and the development of the larval phase, preferably in clean waters, containing organic matter, aquatic vegetation and shading (Manguin et al., 1996a; Rejmankova et al., 1999). Human activity in the Amazon has led to the emergence of new types of breeding sites and larval habitats, such as dams, fish farming tanks, clay pits and deforested areas (Rodrigues et al., 2008; Vittor et al., 2009; Ferreira et al., 2015).

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

The understanding of the relationship between the aquatic phases of the vectors and the environment in which they develop is extremely relevant for malaria control (Molineaux, 1997). The characterization of these new types of breeding sites contributes to knowledge on anopheline population dynamics, biology and ecology, mainly the relationship between environmental factors and the presence of mosquito larvae in these new larval habitats (Tadei and Dutary-Thatcher, 2000; Piyaratne et al., 2005; Ferreira et al., 2015). This knowledge provides information that can influence or determine the location of oviposition and the survival and spatial and temporal distribution of these medically important species. Furthermore, this may also aid in integrated control strategies for these new habitats. Thus, the study sought to characterize the larval habitat of *Anopheles* spp. and to relate environmental parameters to the larval presence of these species, especially the vector of malaria in the Amazon.

## Material and methods

### Study area

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

### Description of the artificial larval habitats

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

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

Dams display characteristics closer to natural environments. They are influenced by springs and formed by man-made barriers used to dam and increase water coverage of a certain area, which

is then used for pisciculture and leisure activities and as a water supply. These areas present average air velocity of 1.5 m/s, 29.4 °C temperature and 80.1% relative humidity. Dams present a vast vegetation cover, with the presence of a ciliary forest, macrophytes and allochthonous material originating from the forest, and display favorable environmental characteristics for the establishment and development of several *Anopheles* and Culicidae species (Fig. 2C).

### *Anopheles* larvae sampling

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

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

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

### Larval habitat water sampling

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

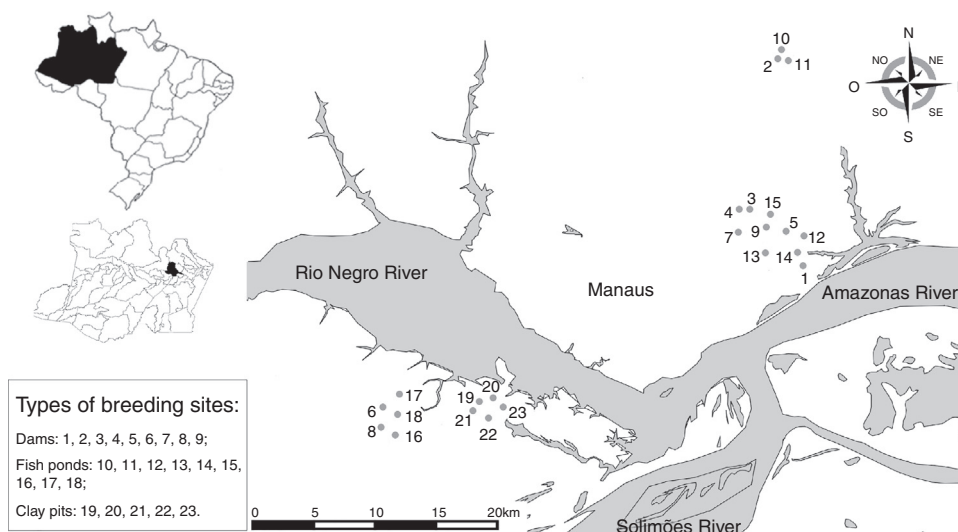
Nitrate and phosphorus determinations were performed using the ion chromatography technique and total suspended solids were evaluated by the gravimetric method. These limnological parameters, alongside their respective values, are recommended by the Brazilian National Council of the Environment – CONAMA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

### Aquatic macrophyte sampling

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

### Structural, environmental larval habitat parameters and malaria cases

Information on the structure of the larval habitats was recorded on a field worksheet created to identify local characteristics, such



**Fig. 1.** Distribution of artificial larval habitats in the metropolitan area of Manaus.



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

as temperature, relative air humidity and wind speed, obtained with a Kestrel 3000 digital thermo-hygro-anemometer. The rainfall index (mm) was obtained directly from the automatic meteorological station installed in Manaus (A101) and requested through the National Meteorological Institute (INMET/Brasília). Positive cases of malaria were obtained from the epidemiological surveillance system and online information (VigWeb, 2017).

#### Data analyses

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

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

## Results

### Anopheles larvae

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

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

### Limnological parameters

Over 90% of the evaluated parameters were within the values described in CONAMA Environmental Resolution no. 357/2005, that establishes values for the maintenance of aquatic life in natural waterbodies (Table 2). Some fish ponds and clay pits presented pH, dissolved oxygen and total phosphorus values below the values recommended by this resolution.

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

### Macrophyte richness

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

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

Anopheles species	Artificial larval habitats <sup>b</sup>																							Total	%
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		
<i>An. triannulatus</i>	156	95	82	139	72	77	123	129	96	43	55	10	42	27	34	19	27	30	5	3	8	4	10	1286	44.9
<i>An. darlingi</i>	36	47	11	17	9	14	13	10	13	39	55	39	37	29	36	71	22	45	3	3	6	5	6	566	19.7
<i>An. nuneztovari</i>	10	23	5	9	11	18	22	15	17	25	22	33	11	29	19	17	30	33	22	17	31	29	16	464	16.2
<i>An. albitarsis s.l.</i>	1	3	9	2	16	5	6	11	5	0	2	2	13	8	15	12	16	9	9	1	2	1	3	151	5.2
<i>An. nimbus</i>	5	74	3	0	0	0	0	0	11	0	0	0	10	3	25	0	0	0	0	0	0	0	0	131	4.5
<i>An. peryassui</i>	0	0	0	0	0	0	11	0	10	0	0	0	8	0	18	26	18	9	5	0	0	6	0	111	3.8
<i>An. braziliensis</i>	2	3	0	4	31	1	0	46	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	92	3.2
<i>An. oswaldoi</i>	0	1	0	0	0	0	0	0	2	0	0	0	0	3	24	0	0	0	3	0	0	0	2	35	1.2
<i>An. evansae</i>	0	0	0	0	0	0	0	2	8	0	0	0	2	1	9	0	0	0	0	0	0	0	0	22	0.7
<i>An. deaneorum</i>	0	0	0	0	3	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0.1
Total	210	246	110	171	142	115	175	213	169	107	134	84	123	100	180	145	113	126	47	24	47	45	37	2,863	100
LIMH <sup>a</sup>	7.0	8.2	3.6	5.7	4.7	3.8	5.8	7.1	5.6	3.5	4.4	2.8	4.1	3.3	6.0	4.8	3.7	4.2	1.6	0.8	1.6	1.5	1.2		

<sup>b</sup> Types of larval habitats: 1–9 dams, 10–18 fish ponds, 19–23 clay pits.

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

Anopheles larval habitats	Temp. (°C)	pH	DO (mg/L)	Cond. (µS/cm)	Turb. NTU	TSS (mg/L)	Nitrate (mg/L)	Phosphorus (mg/L)
1	30.4	6.7	6.1	7.0	1.3	2.2	0.01	0.00
2	28.6	6.2	6.3	10.0	2.8	2.2	1.05	0.02
3	28.4	6.0	6.6	12.0	7.2	10.0	1.35	0.01
4	27.5	6.1	6.0	16.0	3.1	5.2	0.18	0.01
5	32.7	7.3	6.4	7.7	2.6	0.8	0.01	0.00
6	31.3	6.4	8.4	6.0	1.5	6.0	0.04	0.01
7	29.0	6.9	7.2	10.7	2.0	3.6	0.01	0.01
8	30.2	6.5	8.1	11.3	2.2	4.8	0.03	0.01
9	27.2	8.4	7.2	16.2	3.4	11.6	0.09	0.02
10	28.7	5.9 <sup>a</sup>	5.9 <sup>a</sup>	7.4	25.7	9.4	0.03	0.01
11	29.3	5.7 <sup>a</sup>	6.2	6.9	21.6	8.4	0.01	0.01
12	31.1	6.6	4.3 <sup>a</sup>	72.6	26.8	13.0	0.25	0.02
13	29.3	7.4	6.9	88.2	13.2	14.0	0.06	0.01
14	28.9	6.8	6.2	160.6	11.4	16.4	0.05	0.01
15	28.2	6.1	7.1	17.5	3.5	7.0	0.10	0.02
16	30.2	6.3	3.9 <sup>a</sup>	40.5	15.0	20.0	0.85	0.20 <sup>a</sup>
17	31.1	6.5	3.9 <sup>a</sup>	69.6	29.0	19.5	1.02	0.12 <sup>a</sup>
18	30.8	6.7	4.0 <sup>a</sup>	58.4	18.7	23.7	0.93	0.17 <sup>a</sup>
19	30.6	6.3	3.6 <sup>a</sup>	46.1	13.5	18.2	0.09	0.01
20	31.3	6.3	2.3 <sup>a</sup>	97.6	37.3	37.4	0.14	0.10 <sup>a</sup>
21	31.2	7.5	4.0 <sup>a</sup>	52.5	24.4	69.6	0.30	0.09 <sup>a</sup>
22	31.4	6.1	3.9 <sup>a</sup>	91.3	32.1	58.3	0.06	0.12 <sup>a</sup>
23	30.9	7.2	2.6 <sup>a</sup>	77.9	29.9	25.1	0.19	0.01
<sup>a</sup> CONAMA No. 357/2005	–	6.0–9.0	>6.0	–	<40.0	<500	<10.0	<0.020

<sup>a</sup> Values outside the standards recommended by the current environmental resolution.

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

Macrophytes	Dams	Fish ponds	Clay pits
<i>Utricularia</i> sp.	X	X	X
<i>Brachiaria</i> sp.	X	X	–
<i>Pistia</i> sp.	X	X	–
<i>Oriza</i> sp.	X	–	–
<i>Xyris</i> sp.	X	–	–
<i>Cyperus</i> sp.	X	X	–
<i>Urosphata</i> sp.	X	–	–
<i>Salvinia</i> sp.	X	X	–
<i>Cabomba</i> sp.	X	X	–
<i>Eichhornia</i> sp.	X	–	–
<i>Marsilea</i> sp.	X	–	–
<i>Eleocharis</i> sp.	X	X	X
<i>Nymphaea</i> sp.	–	X	–

#### Relationship between environmental parameters and Anopheles

The canonical correlation analysis (CCA) indicated significant positive correlations between *An. albitarsis*, *An. peryassui* and *An. nuneztovari* with conductivity ( $r=0.4623$ ,  $p=0.021$ ), total suspended solids ( $r=0.7656$ ,  $p=0.001$ ) and phosphorus ( $r=0.7033$ ,

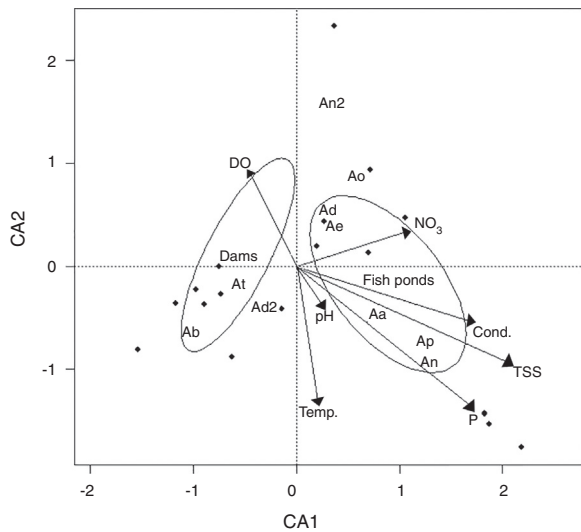
$p=0.003$ ) in fish tanks ( $r=0.2609$ ,  $p=0.01$ ). Species *An. triannulatus* and *An. braziliensis* presented positive correlations with the dam larval habitat ( $r=0.2609$ ,  $p=0.01$ ), but not with any limnological parameter. The other parameters and anopheline species were not positively correlated ( $p>0.05$ ) (Fig. 3).

## Discussion

### Artificial larval habitats and entomological parameters

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

Positive cases peaks of autochthonous malaria in Manaus occurred at the dry season peak on the second half of two

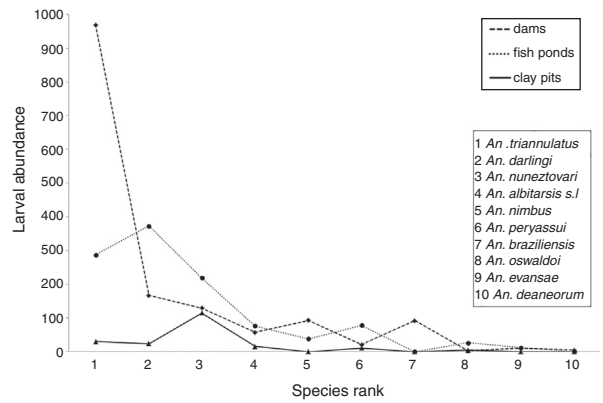


**Fig. 3.** Ordering diagram of the canonical correlation analysis (CCA) between environmental factors “limnological parameters” and larval habitat type with *Anopheles* species: At (*Anopheles triannulatus*); Aa (*Anopheles albitarsis s.l.*); Ad (*Anopheles darlingi*); An (*Anopheles nuneztovari*); Ao (*Anopheles oswaldoi*); Ap (*Anopheles peryassui*); Ab (*Anopheles braziliensis*); An2 (*Anopheles nimbus*); Ad2 (*Anopheles deaneorum*); Ae (*Anopheles evansae*); DO (dissolved oxygen); NO<sub>3</sub> (nitrate); pH (hydrogenionic potential); Temp (temperature); Cond (electrical conductivity); P (phosphorus); TSS (total suspended solids).

consecutive years (Fig. 4). By October 2017, there were 3030 cases and one death, which indicates a large increase in relation to previous years (VigWeb, 2017). These artificial larval habitats present a relevant role in these cases of malaria regardless of the rivers level and amount of rainfall in the region, since they are active throughout the year, influencing the abundance of the vector mosquito *An. darlingi* in the dry season. However, in natural environments rainfall availability is a limiting factor in the abundance of mosquitoes (Fillinger et al., 2004), and also higher occurrences of *An. darlingi* are found in the intermediate (winter-summer) period in the Amazon (Tadei et al., 1993). And the fast increase in the abundance of these mosquitoes may hinder the development of effective vector.

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

Some studies already cover these types of sites in the Amazon (Rodrigues et al., 2008; Vittor et al., 2009; Ferreira et al., 2015; Reis et al., 2015), indicating the presence of the malaria vector in the region. Environments like lagoons are more favorable habitats for the development of several *Anopheles* species (Rejmankova et al., 1999; Rubio-Palis et al., 2005), mainly due to the presence of



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

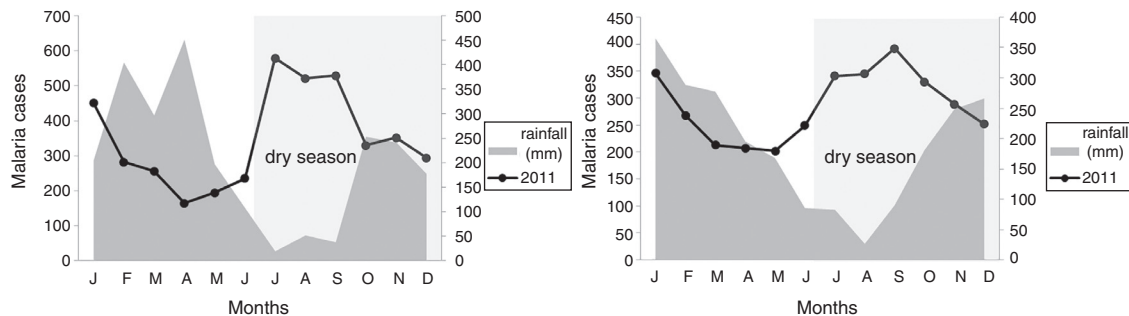
macrophytes, creating a microhabitat that serves as a refuge against predators, favoring oviposition (Orr and Resh, 1989; Orr and Resh, 1992). This was observed in the investigated dams in the present study, since they share similar characteristics to natural environments, including the presence of macrophytes, water quality and shading.

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

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

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

Similar results regarding the distribution of the most frequent species observed herein and the larval density of anophelines have been observed in other endemic malaria areas (Moreno et al., 2000; Berti-Moser et al., 2008). Some studies have recorded the presence



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

of immature *An. triannulatus* and *An. darlingi* in different larval habitats (Rubio-Palis et al., 2005; Moreno et al., 2007; Ferreira et al., 2015). This behavior, also observed herein, may serve as an indication for the presence of these species in similar breeding sites, indicating their coexistence.

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

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

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

#### Limnological parameters

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

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

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

The presence of *Anopheles* was recorded even in breeding sites displaying low plant cover and high temperatures, demonstrating the versatility of this species regarding abiotic parameters. The high temperatures observed in turbid water pools increase

evaporation rates, reducing the permanence of these larval habitats in the environment, leading to the acceleration of the larval development (Paaijmans et al., 2008), especially in clay pits.

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

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

#### Relationship between environmental parameters and *Anopheles*

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

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

In malaria vector breeding sites in Sri Lanka, only abiotic factors and structural characteristics of the breeding site indicate *Anopheles* preference for habitat types, not limnological parameters (Piyaratne et al., 2005). However, a positive correlation was observed between limnological parameters and larval habitat type in the artificial sites evaluated herein. With a clear preference of certain species for more natural habitats while others prefer nutrient-rich sites with more artificial features. The positive and negative relation with dissolved oxygen values in larval habitats has been described as a determinant factor for the larval presence of both anophelines and other Culicidae (Pinault and Hunter, 2012; Devi et al., 2015; Kudom, 2015). This limnological parameter was

shown to be an important variable in the correlation analysis in dams, indicating a more natural larval habitat, with high oxygen concentrations when compared to the other habitats.

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

## Conclusion

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

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## Authors' contribution

ANA, HBC and WPT conceived and designed the experiments; ANA and FASF performed the experiments and analyzed the data; HBC and WPT contributed reagents/materials/analysis tools; ANA wrote the paper.

## Conflicts of interest

The authors declare no conflicts of interest.

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