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Research Article / Artículo de Investigación

Limnological variables associated with the presence of *Anopheles* Meigen, 1818 (Diptera: Culicidae) larvae in breeding sites in Amazonas, Brazil

Variables limnológicas asociadas a la presencia de larvas de *Anopheles* Meigen, 1818 (Diptera: Culicidae) en criaderos de Amazonas, Brasil

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Abstract. The objective of this study was to correlate environmental characteristics (biotic and abiotic) of different breeding site types with the richness and abundance of anophelines in the metropolitan region of Manaus, Brazil. *Anopheles* larvae and biotic and abiotic parameters were collected in artificial breeding sites, where we demonstrated that the dams presented characteristics of natural environments, with good water quality, shaded areas, and the presence of macrophytes, positively influencing the larval abundance of anophelines. The fish ponds are in the process of transitioning from a natural to an altered environment, followed by breeding sites classified as clay pits (anthropized). Macrophyte richness was higher in dams and fish ponds, being responsible for creating microhabitats for larvae and the structure of breeding sites. Ten anopheline species were identified, totaling 1,186 individuals, with an abundance of *An. triannulatus* (Neiva & Pinto, 1922), *An. albitarsis* Lynch-Arribálzaga, 1878, *An. nuneztovari* Gabaldón, 1940 and *An. darlingi* Root, 1926. Malaria vectors in the region were present in all collection sites. A good part of the physicochemical parameters were in agreement with the current environmental resolution, mainly in less altered environments. Another strong correlation was observed between anophelines and some limnological parameters, which may be an indicator of the presence of these Culicidae in the environments.

Key words: *Anopheles darlingi*; larval habitat; malaria vector; mosquitoes; water quality.

Resumen. El objetivo de este estudio fue correlacionar las características ambientales (bióticas y abióticas) de diferentes tipos de criaderos con la riqueza y abundancia de anofelinos en la región metropolitana de Manaus, Brasil. Fueron recolectados inmaduros y parámetros bióticos y abióticos en criaderos artificiales, donde se demuestra que las presas presentaban características de ambientes naturales, con buena calidad del agua, áreas sombreadas y presencia de macrófitas, influenciando positivamente la abundancia larval de anofelinos. Los estanques piscícolas están en proceso de transición de un medio natural a un medio alterado, seguidos de los criaderos clasificados como fosas de arcilla (antropizados). La riqueza de macrófitos fue mayor en las presas y charcas, siendo responsables de la creación de un microhábitat para las larvas y de la estructura de los lugares de cría. Se identificaron diez especies de anofelinos, con un total de 1.186 individuos, con abundancia de *An.*

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triannulatus (Neiva y Pinto, 1922), *An. albitarsis* Lynch-Arribálzaga, 1878, *An. nuneztovari* Gabaldón, 1940 y *An. darlingi* Root, 1926. El vector de la malaria en la región estuvo presente en todos los lugares muestreados. Buena parte de los parámetros fisicoquímicos concordaban con la resolución ambiental actual, principalmente en los ambientes menos alterados. También se observó una fuerte correlación entre los anofelinos y algunos parámetros limnológicos, lo que puede ser un indicador de la presencia de estos Culicidae en los ambientes.

Palabras clave: *Anopheles darlingi*; calidad del agua; hábitat larvario; mosquitos; vector de la malária.

Introduction

The Amazon region is currently the target of intense environmental changes, due to human actions, both by the installation of new ventures (opening of roads, damming, fish farming ponds, mining, and pottery holes) and by the irregular expansion of cities. These actions influence the emergence of new artificial breeding sites (*e.g*., fish ponds, dams, and clay pits) (Ferreira *et al*. 2015; Arcos *et al*. 2018a). These activities introduce changes in the ecosystem, affecting an integrated organism-environment complex and in the biology of diseases, including malaria (Tadei *et al.* 1988).

The main vector of malaria in the Amazon region is *Anopheles* (*Nyssorrhynchus*) *darlingi* Root, 1926, while other species are related as secondary transmitters of *Plasmodium* that cause human malaria, including *Anopheles aquasalis* Curry, 1932 and *Anopheles albitarsis* Lynch-Arribálzaga, 1878 (Rubio-Palis *et al.* 1992; Forattini 2002; Tadei *et al.* 2017). The region has geographical and ecological characteristics that are highly favorable for the interaction of *Plasmodium* with anopheline vectors, constituting an area of high and medium risk of infection (Silveira and Rezende 2001; Recht *et al.* 2017).

Immatures develop in the aquatic environment, in natural and artificial, permanent, temporary, and of various qualities, volumes, and sizes (Tadei 1993; Consoli and Lourenço-de-Oliveira 1994; Tadei *et al.* 1998; Barbosa and Scarpassa 2023), preferably in habitats with clean water, where remains of organic matter from local vegetation, submerged vegetation and shading are present. However, the flooding of rivers and high volumes of rain provide the emergence of numerous breeding sites, such as puddles, ditches, and waterlogged fields (Manguin *et al.* 1996; Rejmankova *et al.* 1999).

As mosquito larvae develop in an aquatic environment, they are subject to temperature variations in the aquatic habitat and other factors. Light and temperature, for example, exert direct effects on the metabolism and development of larvae and, indirectly, have a fundamental role in providing favorable conditions for the proliferation of algae and other organisms used in the feeding of Culicidae (Forattini 1962; Bergo *et al.* 1990). In addition, environmental factors such as forest cover also, directly, and indirectly, affect the composition of the vector and non-vector mosquito communities in the Amazon (Arcos *et al.* 2021), jointly the land use change can alter the risks of emerging zoonotic disease (Roque *et al.* 2023).

The presence of vegetation in aquatic environments is one of the main factors linked to the structuring of habitats occupied by aquatic animal communities. In addition to their role in nutrient dynamics, macrophytes contribute to increasing the structural heterogeneity of habitats, affecting biological diversity, interspecific relationships, and system productivity, as well as providing micro-habitat and natural shelter for larvae (Forattini *et al.* 1994; Thomas and Bini 2003). Therefore, the quali-quantitative data followed the diversity and structure that make up the anopheline larval habitats in the Amazon region (Lima *et al.* 2021).

The relationship of environmental variables with the presence of anophelines provides us with important data on the bioecology of vector and non-vector mosquitoes, especially the malaria vector in the region. Therefore, the objective of this study was to relate the water quality of breeding sites with the larval composition of anophelines in different regions in the city of Manaus, Amazonas, Brazil.

Material and Methods

Sampling was carried out in the peri-urban areas of Manaus between September 2012 and May 2013, in artificial breeding sites located in the region of the AM-070 highway (1, 2, 8, 9, 10) and Puraquequara (3, 4, 5, 6, 7) (Fig. 1).

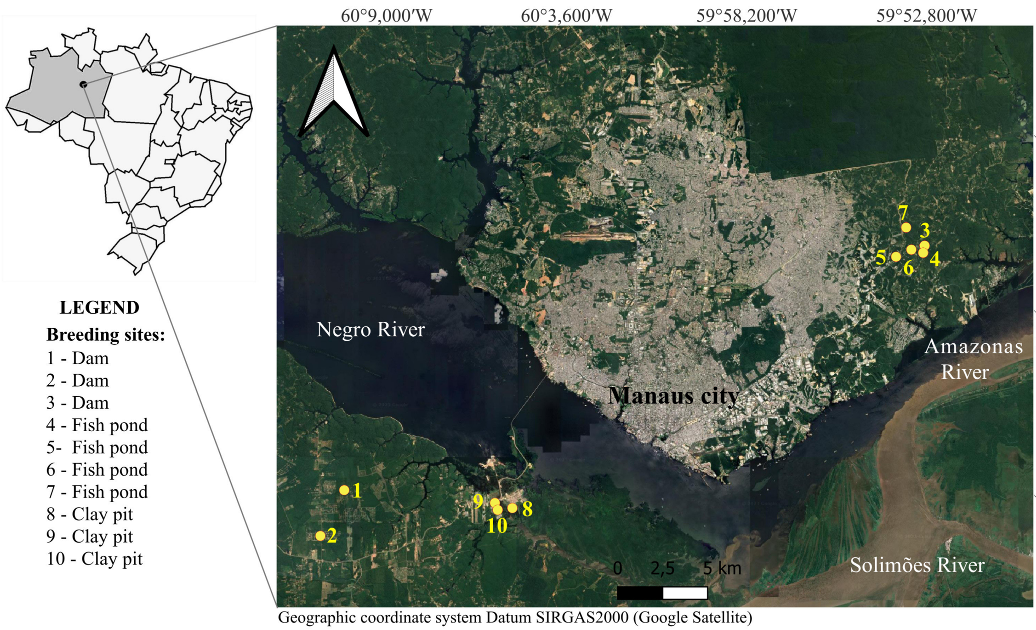

Figure 1. Distribution of breeding sites in the peri-urban area of the metropolitan region of Manaus, Amazonas, Brazil. / Distribución de los criaderos en el área periurbana de la región metropolitana de Manaus, Amazonas, Brasil.

The breeding sites selected for the study are classified as artificial, called: dams (B), fish ponds (T), and clay pits (P). Its characterization with information on structural characteristics is described in a study carried out by Arcos *et al*. (2018a). Limnological variables were collected in all breeding sites, and the water samples were stored in 500 mL flasks, and taken to the Environmental Chemistry Laboratory - INPA, for filtering, drying, and weighing the total solids in suspension on cellulose paper, using the gravimetric method (Apha 1985). To analyze the concentration of phosphate and nitrate, the water sample was fixed with Tymol, and determined through ion chromatography techniques in the DIONEX device (Apha 1985; Nguyen *et al*. 2012). Some parameters such as water temperature, pH, dissolved oxygen and electrical conductivity were measured on site with the aid of portable equipment (Orion pH 290A+, YSI Dissolved oxygen, and VWR "EC METER" 2052).

The aquatic macrophytes present in the breeding sites were separated according to their classification (emerging, submerged, and floating). The fresh material was sent to the laboratory for the determination of the species richness present. The botanical material was identified by comparing previously identified specimens of a Collection (MAUA Group and INPA/Max-Planck cooperation) and specialized literature (Stodola 1967; Pott and Pott 2000; Lorenzi 2000; Souza and Lorenzi 2005).

The larvae were collected with a standard 350 mL dipper and a one-meter cable to reach the areas around the breeding sites, in the water column, with a sampling effort of 30 minutes of collection for each collector, at each point, to calculate the larvae index per man-hour – LIMH/ILHH (Tadei *et al.* 2007).

The larvae were removed with the aid of a Pasteur pipette, stored in Falcon tubes and transported to the malaria and dengue laboratory at INPA for breeding. They were separated according to the respective stages of development and created in the laboratory, in trays containing water. These immatures were fed with macerated fish food (TetraMin Marine Saltware®). The specimens were kept under controlled conditions of feeding, temperature (26 ± 2 °C), relative humidity above 85%, and photoperiod (12 h) (Oliveira *et al*. 2012; Arcos *et al*. 2018a).

Mosquitoes were identified using the dichotomous keys proposed by Gorham *et al.* (1967), Faran (1980), Faran and Linthicum (1981), and Consoli and Lourenço-de-Oliveira (1994). The larvae index per man-hour – LIMH/ILHH was used to measure the larval density, and the index takes into account the sampling effort, which is important because it also allows locating points of greater receptivity for anopheline production (Tadei 2001). Simple regression analysis was used to verify the relationship between macrophyte richness and anopheline abundance and richness. The program used was STATISTICA ® 8.0 and the results were considered when the significance level was ($p < 0.05$) (StatSoft, Inc. 2007).

Principal component analysis (PCA) is a mathematical technique of multivariate analysis, which allows investigations with a large number of available data. It also makes it possible to identify the measures responsible for the greatest variations between the results, without significant loss of information, transforming an original set of variables into another set: the principal components (PC) of equivalent dimensions. Cluster and similarity analysis was carried out using Euclidean distance. We sought to verify the relationship of the limnological parameters with the types of breeding sites and the presence of anophelines, and the statistical program MINITAB ® Release 14 was used for the analysis.

Results and Discussion

We collected 1,185 specimens, and breeding sites B1, B3, and T7 had the highest abundance. The most abundant species were *Anopheles triannulatus*, *Anopheles nuneztovari*, and *Anopheles darlingi*, the latter of which is the main vector of malaria in the Amazon region and was present in all the breeding sites. The breeding sites had a total richness of nine species, and the fish ponds stood out in relation to the other types of breeding sites (Tab. 1, Fig. 1). The B1, B2, B3, T6 and T7 breeding sites had the highest rate of larvae per man-hour.

Table 1. Anopheline larval abundance in different breeding sites distributed in the metropolitan region of Manaus, Amazonas. / Abundancia de larvas de anofelinos en diferentes criaderos distribuidos en la región metropolitana de Manaus, Amazonas.

Species	B1	B2	B3	T4	T5	T6	T7	P8	P9	P10	Total
An. triannulatus (Neiva & Pinto, 1922)	138	96	113	34	27	29	20	4	9	12	482
An. darlingi Root, 1926	24	13	5	57	41	43	46	2	5	3	239
An. nuneztovari Gabaldón, 1940	5	0	0	6	8	17	10	62	55	33	196
An. braziliensis (Chagas, 1907)	10	8	46	0	0	7	0	0	0	0	71
An. albitarsis Lynch-Arribálzaga, 1878 *s.l.*	0	6	0	13	8	10	15	2	6	1	61
An. peryassui Dyar & Knab, 1908	7	5	1	2	0	5	8	6	8	13	55

An. nimbus (Theobald, 1902)	0	0	0	10	3	11	15	0	0	0	39
An. evansae (Brèthes, 1926)	0	1	2	2	1	8	9	0	0	0	23
An. oswaldoi (Peryassú, 1922)	0	0	0	0	3	2	14	0	0	0	19
Total	**184**	**129**	**167**	**124**	**91**	**132**	**137**	**76**	**83**	**62**	**1,185**
LIMH/ILHH*	**3.0**	**2.1**	**2.7**	**2.0**	**1.5**	**2.2**	**2.3**	**1.2**	**1.3**	**1.0**	

*Larvae index per man-hour (LIMH/ILHH). B = Dam; T = Fish pond; P = Clay pit.

The diversity of *Anopheles* species is directly related to the types of breeding sites that exist and which can favor the development of the mosquito life cycle (Gallardo and Povoa 2010). In this sense, we also identified this profile, especially among natural breeding sites with good water quality, with the abundance and richness of species, including the malaria vector *An. darlingi* (B1, B2, B3, T4, T5, T6, T7) (Tab. 1). The use of environmental parameters to characterize a habitat is fundamental to understanding the entire system that surrounds the habitat, thus providing information on bioecology and control measures. Some studies in the region have been developing activities in these types of breeding sites, mainly in vector control strategies and characterization of the aquatic habitat using biotic and abiotic variables (Rodrigues *et al.* 2008; Ferreira *et al.* 2015; Arcos *et al.* 2018b, c).

Most of the specimens of *An. darlingi* and *An. triannulatus* were collected in environments with more natural characteristics (without alterations and typical of Amazonian aquatic environments), as observed in this study and by Galardo *et al.* (2009) in breeding sites in the state of Amapá and by Arcos *et al.* (2018a) in Manaus. Some anophelines known as secondary vectors that coexist with *An. darlingi* can transmit malaria. Studies have reported the possibility of *An. triannulatus*, *An. nuneztovari*, and *An. oswaldoi* being secondary vectors of malaria in the Amazon (Arruda *et al.* 1986; Oliveira-Ferreira *et al.* 1990; Branquinho *et al.* 1996; Saraiva *et al.* 2018). It is worth noting that all of these species were present in the breeding sites studied in Manaus and are generally common in the region.

The fish ponds showed the highest larval density (LIMH/ILHH), followed by the dams and lastly the clay pits. It was observed that these values followed the diversity and structure of the breeding site, such as the presence or absence of macrophytes, limnological variables, and others (Tab. 1). According to Tadei (2001), the larvae index per man-hour also makes it possible to locate points of greatest receptivity for anopheline production.

In general, few studies have been carried out to analyze the biotic and abiotic structure of breeding sites in the Amazon. A recent study with *An. darlingi* collected from ponds and dams in the middle Solimões region of Amazonas, sought to identify the microbiota associated with the mosquito and the aquatic environment of the breeding sites, in an attempt to understand their interactions and potential uses (Mosquera *et al.* 2023). Another region affected by the proliferation of the malaria vector is in indigenous areas of the State, with few studies on the larval bionomics of anophelines. Furthermore, these regions are highly heterogeneous in terms of the occurrence of anophelines, especially *An. darlingi* and other secondary species. The breeding sites that showed the highest larval density of the vector were lakes associated directly with the main river (Sánchez-Ribas *et al.* 2017). In this sense, the presence of the malaria vector in the Amazon region points to the epidemiological importance of these environments, especially artificial breeding sites classified in this study (dams, fish ponds and clay pits). These habitats are scattered throughout the Amazon region and become an important point for vector control and surveillance strategies.

The structure of the habitat was a determining factor in the composition of anopheline species. Breeding sites classified as dams showed characteristics of environments with low environmental impact, favoring the presence of *An. triannulatus* and *An. darlingi*. Fish ponds had a moderate anthropogenic impact and the most frequent species were *An. albitarsis* and *An. darlingi*, where the abundance of the malaria vector was mainly associated with habitats close to human residences, as it is a mosquito with an anthropophilic habit. The clay pits are more recent breeding sites, with high levels of anthropogenic modification, turbid water, and the presence of *An. nuneztovari* and *An. peryassui* larvae (Fig. 2). Some studies have noted the importance of these breeding sites in the region and applying vector control techniques in the field, semi-field, and laboratory, using biological and chemical larvicides. They are also investigating the effects on non-target entomofauna (Rodrigues *et al.* 2008; Ferreira *et al.* 2020).

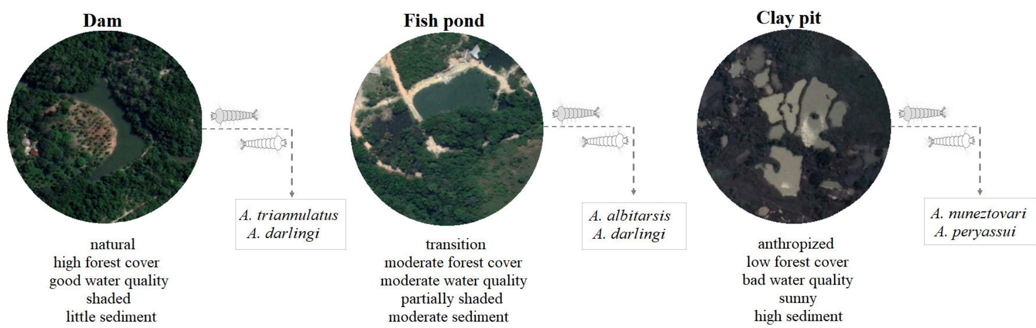

Figure 2. Observed relationship between larval habitat characteristics and anopheline composition in breeding sites in the metropolitan area of Manaus, Amazonas. / Relación observada entre las características del hábitat larval y la composición de anofelinos en sitios de reproducción en el área metropolitana de Manaus, Amazonas.

Among the breeding sites studied, it is worth highlighting one of the most recent, called clay pits (altered) (Fig. 2). Due to the removal of clay, holes are formed and over time receive the action of rainfall and the hydrological regime, where they gradually fill up and become favorable for larval development, mainly due to the emergence of marginal vegetation and the growth of microalgae. In general, the quantity of larvae in the breeding sites depends directly on the conditions for their development. According to Tadei *et al.* (1998), the required time for the natural succession process and the establishment of the water body in these new breeding habitats is around three years, and consequently, the colonization by anophelines (*e.g.*, clay pits). Another important point would be vector control combined with the creation of these fish ponds in the Amazon. These habitats have become the main artificial breeding sites for anophelines, influencing the increase in the vector in the region and consequently in malaria cases (Barbosa and Scarpassa 2023).

Twelve genera of macrophytes were identified, including *Utricularia* L., *Brachiaria* (Trin.) Griseb., *Pistia* L., *Oriza* L., *Xyris* Gronov. ex L., *Cyperus* L., *Urospatha* Schott, *Salvinia* Ség., *Cabomba* Aubl., *Eichhornia* Kunth, *Marsilea* L., and *Eleocharis* R.Br. The breeding sites with the highest richness were B1 and T6, both with seven genera, followed by breeding site B3 with six genera. The lowest richness was found in breeding sites T5 and P9, with one and two genera respectively (Tab. 2). The hypothesis that breeding sites with higher macrophyte richness influence the abundance and richness of anopheles was not corroborated ($r2= 0.0033$, $p= 0.8741$ / $r2= 0.0001$ $p= 0.9762$). However, this behavior can be explained by other groups of variables and also by the abundance of macrophytes. Therefore, no significant relationship was observed between the variables studied $p> 0.05$.

Table 2. Presence and absence of aquatic macrophytes in breeding sites in the metropolitan area of Manaus, Amazonas. / Presencia y ausencia de macrófitos acuáticos en criaderos del área metropolitana de Manaus, Amazonas.

Genera	B1	B2	B3	T4	T5	T6	T7	P8	P9	P10
Utricularia L.	X	-	X	-	-	X	X	X	X	X
Brachiaria (Trin.) Griseb.	X	X	X	X	-	X	-	X	-	-
Pistia L.	X	-	-	-	-	-	X	-	-	-
Oryza L.	X	-	-	-	-	-	-	-	-	-
Xyris Gronov. ex L.	X	-	-	-	-	X	-	-	-	-
Cyperus L.	X	-	X	X	-	X	-	-	-	-
Urospatha Schott	X	-	X	-	X	-	-	-	-	-
Salvinia Ség.	-	X	X	X	-	X	X	-	-	-
Cabomba Aubl.	-	X	X	-	-	-	-	-	-	X
Eichhornia Kunth	-	-	-	-	-	X	-	-	-	-
Marsilea L.	-	-	-	-	-	X	-	-	-	-
Eleocharis R.Br.	-	-	-	-	-	-	-	X	X	X

Three categories of macrophytes were found in all the breeding sites studied: floating, submerged, and emergent. Manguin *et al.* (1996) identified emergent macrophytes as the most common in breeding sites and with a positive association with the presence of larval mosquitoes. Dams (B1 to B3) and fish ponds (T4 to T7) showed greater richness, however, there was no statistically significant relationship between macrophyte richness and the abundance and larval richness of *Anopheles* (Tab. 2). According to Forattini (2002), depending on the type and quality of the breeding site, the number of mosquito species can vary from place to place. In this sense, the breeding sites with the highest macrophyte richness had the highest richness and abundance of *Anopheles* larvae (Tabs. 1, 2). According to Forattini (1962) and Esteves (1998), aquatic macrophytes form a relatively stable and safe microhabitat for anopheline immatures in relation to predators, providing a shaded area, as well as helping to absorb pollutants and excess organic substances in the environment.

The pH values ranged from 6.1 to 8.4 ± 0.6 in the breeding sites studied, with the highest value observed in T6 and the lowest in T7. All the values observed were within the standards stipulated by CONAMA Environmental Resolution n° 357/2005 (Tab. 3). Water temperature did not vary greatly between habitats, with the highest values found in breeding sites B1 (31.3 °C) and P9 (31.1 °C). The lowest values were measured in breeding sites T6 (27.2 °C) and T7 (28.2 °C). Electrical conductivity varied noticeably between the breeding sites, with values ranging from 6.0 µS/cm (B1) to 160.6 µS/cm (T5) ± 48.7 µS/cm (Tab. 3).

The clay pits showed the lowest values of dissolved oxygen in the breeding sites, P8 (3.9 mg/L), P9 (2.9 mg/L), and P10 (4.0 mg/L), being outside the standards of environmental resolution n° 357/2005 from the CONAMA's environmental resolution of Brazil. The highest values were found in dams B1 (8.4 mg/L) and B3 (8.1 mg/L), which have the characteristics of natural environments. The values for total suspended solids and turbidity were below the limit stipulated by the environmental resolution in all the breeding sites. The nutrients phosphate and nitrate were present in higher concentrations in the breeding sites classified as clay pits, due to the accumulation of sediment, however, they did not exceed the standard limit (Tab. 3).

Table 3. Characterization of limnological parameters in breeding sites distributed in the metropolitan region of Manaus, Amazonas. / Caracterización de parámetros limnológicos en criaderos distribuidos en la región metropolitana de Manaus, Amazonas.

Larval habitat	pH	Temp. °C	Cond. µS/cm	O² mg/L	TSS mg/L	Turbidity NTU	Nitrate ppm	Phosphate ppm
B1	6.4	31.3	6.0	8.4	6.0	1.5	0.04	0.02
B2	6.9	29.0	10.7	7.2	3.6	2.0	0.01	0.01
B3	6.5	30.2	11.3	8.1	4.8	2.2	0.03	0.07
T4	7.4	29.3	88.2	6.9	14.0	13.2	0.06	0.03
T5	6.8	28.9	160.6	6.2	16.4	11.4	0.05	0.01
T6	8.4	27.2	16.2	7.2	11.6	3.4	0.09	0.02
T7	6.1	28.2	17.5	7.1	7.0	3.5	0.10	0.04
P8	6.3	30.2	40.5	3.9	20.0	15.0	0.85	0.50
P9	6.5	31.1	69.6	2.9	19.5	29.0	1.02	0.61
P10	6.7	30.8	58.4	4.0	23.7	18.7	0.93	0.30
standard deviation	0.6	1.3	48.7	1.9	7.1	9.1	0.42	0.22
CONAMA n° 357/2005	6 - 9	-	-	> 6.0	< 500	< 40	< 10	-

The electrical conductivity of water is one of the most important variables in limnology, as it synthesizes information about the presence of dissolved ions (Mucci 2008). High values of total suspended solids, turbidity, and nutrients in breeding sites such as fishponds and clay pits increase the levels of electrical conductivity in these environments. According to Okogun *et al.* (2003), breeding sites with an almost neutral pH of between 6.8 and 7.2 are ideal for weakening *Anopheles* egg shells. Furthermore, Kengluecha *et al.* (2005) reported that environmental variables, in addition to contributing to the environment, can also determine the larval abundance of *Anopheles* in breeding sites. Becker (2008) points out that the higher the temperature, the faster the life cycle of the mosquito, influencing the progression of generations and the size of the vector population. High temperatures in murky water breeding sites cause faster evaporation, reducing the permanence of these habitats and *Anopheles* will have a shorter time to reach the adult stage (Paaijmans *et al.* 2008). More generally temperature is a key parameter to explain the distribution of *Anopheles* mosquitoes (Rhodes *et al.* 2022).

These same observations were recorded by Tadei *et al.* (1993, 1998), in studies on anopheline breeding sites in blackwater rivers in Amazonas. It was found that *An. oswaldoi* and *An. mediopunctatus* are species that show ample tolerance to variations in the pH of the breeding site and that the types of algae presents do not limit their occurrence (Tadei *et al.* 1993, 1998). Rejmankova *et al.* (1993) found that water temperature and dissolved oxygen had little correlation with larval occurrence in breeding sites. However, Shililu *et al.* (2003) found the opposite to be true, with a significant effect on larval density in breeding sites, varying on average from 19.7 to 28.8 °C.

According to Arcos *et al.* (2018b), because natural breeding sites are more balanced than other types, they facilitate the establishment and development of anopheline larvae and other macroinvertebrates. In addition, breeding sites with excessive amounts of algae (*e.g.*, filamentous algae, cyanobacteria) end up reducing the larval abundance and can make it difficult for them to move and breathe. The use of environmental indicators to assess water quality is an important tool for tracing potential environmental impacts and characterizing areas (e.g., maintenance of aquatic life, environmental monitoring, vector bioecology) (Rufalco-Moutinho *et al.* 2016; Falcão *et al.* 2021; Arcos and Cunha 2022). Limnological variables are key to understanding the relationship between biotic and abiotic variables in natural and artificial environments, especially in the Amazon, which has been affected by increased deforestation, urbanization, and land use (Vittor *et al.* 2009; Reis *et al.* 2015; Arcos *et al.* 2021).

The similarity analysis formed three clusters, the first was made up of breeding sites with more natural characteristics (B1, B2, B3, T7), the second group are breeding sites in transition (T4, T5, T6), and the third group was made up of more altered breeding sites (P8, P9, P10) (Fig. 3A). Principal component analysis (PCA) showed that axis 1 explained 59.0% and axis 2 25.6%, together explaining 84.6% of the variation in physicochemical data in the habitats (Fig. 3B). The habitats were grouped according to their limnological characteristics, with points B1, B2, and B3 grouped together in environments with high levels of dissolved oxygen in the water. In addition, points T4 to T6 (fishponds) were grouped together as environments with high pH values, while the pottery ponds (P8, P9, P10) were grouped together as having high values for temperature, nutrients, and sediment in the water.

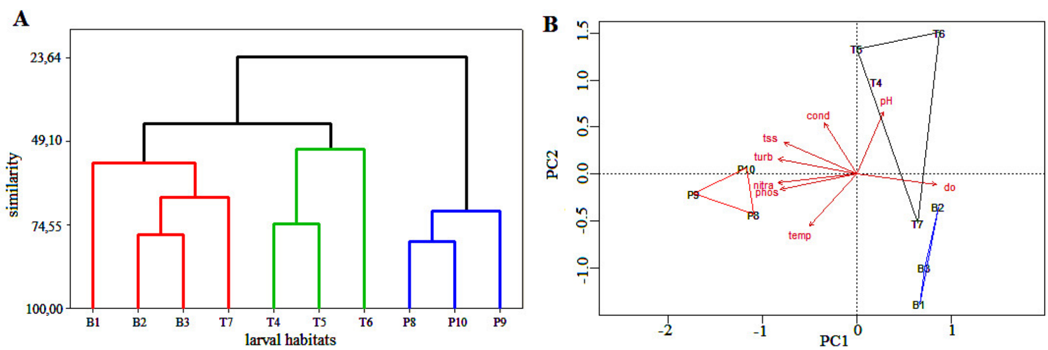

Figure 3. Similarity dendrogram of the larval habitats **(A)**, and Principal Component Analysis – PCA **(B)** involving the limnological variables from artificial larval habitats of anophelines. Abbreviations: tss (total suspended solids); turb (turbidity); cond (conductivity); do (dissolved oxygen); temp (temperature); nitra (nitrate); phos (phosphate); larval habitats: B1, B2, B3, T4, T5, T6, T7, P9, P9, P10. / Dendrograma de similitud de los hábitats larvarios **(A)**, y Análisis de Componentes Principales - ACP **(B)** que incluye las variables limnológicas de los hábitats larvarios artificiales de anofelinos. Abreviaturas: tss (sólidos suspendidos totales); turb (turbidez); cond (conductividad); do (oxígeno disuelto); temp (temperatura); nitra (nitrato); phos (fosfato); hábitats larvarios: B1, B2, B3, T4, T5, T6, T7, P9, P9, P10.

Principal component analysis (PCA) showed the relationship between anopheline species and some limnological variables, where four clusters were formed with a strong relationship. The *An. triannulatus* species is positively influenced by dissolved oxygen (DO) and inversely influenced by the other abiotic factors (Fig. 4A). Electrical conductivity and total suspended solids (STS) were related to the presence of *An. albitarsis* in the breeding sites (Fig. 4B). Another relationship observed was the species *An. nuneztovari* positively correlated with phosphate and nitrate (Fig. 4C), and *An. peryassui* with a direct positive correlation between turbidity and STS (Fig. 4D). The other species such as *An. oswaldoi*, *An. darlingi*, *An. nimbus*, and *An. evansae* showed no relationship with the other limnological variables.

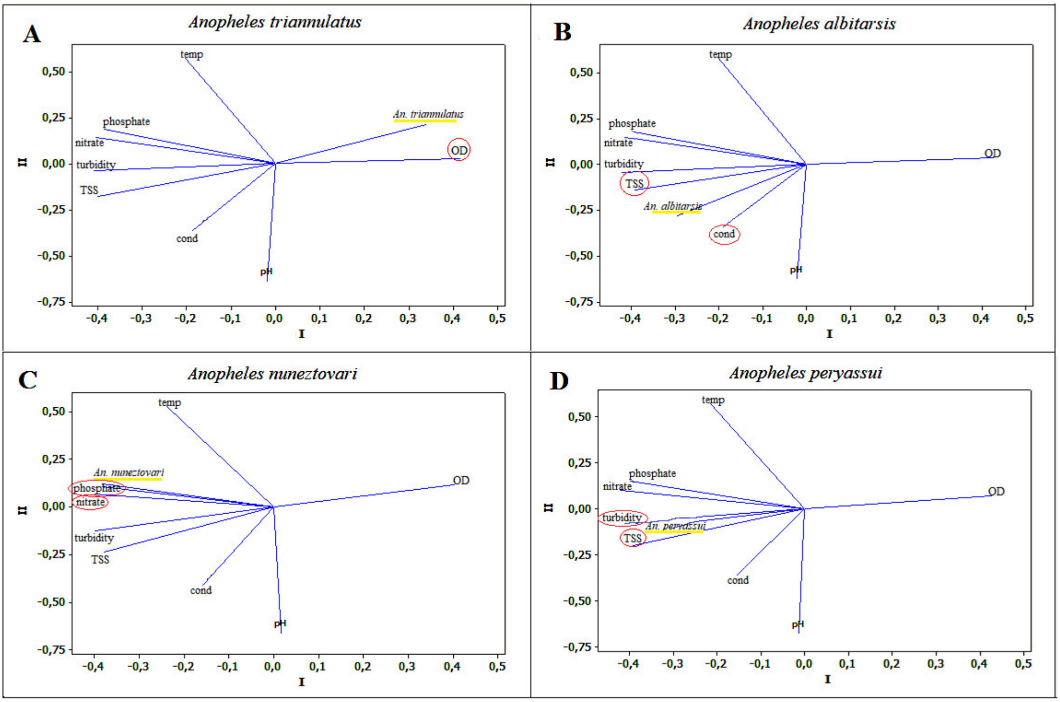

Figure 4. Principal Component Analysis (PCA) between limnological variables and anopheline composition in larval habitats. pH; turbidity; nitrate; phosphate; TSS (total suspended solids); cond (conductivity); OD (dissolved oxygen); temp (temperature). / Análisis de Componentes Principales (ACP) entre variables limnológicas y composición de anofelinos en hábitats larvarios. pH; turbidez; nitrato; fosfato; SST (sólidos suspendidos totales); cond (conductividad); OD (oxígeno disuelto); temp (temperatura).

Principal component analysis showed a strong positive relationship between *An. triannulatus*, *An. albitarsis*, *An. nuneztovari*, and *An. peryassui* with dissolved oxygen, conductivity, total suspended solids, phosphate, nitrate, and turbidity. This pattern can serve as a determining factor in the presence of these species, especially in these new environments classified as artificial breeding sites. Studies on natural breeding sites have indicated that physical and chemical factors determine the occurrence of anopheline species in breeding sites (Berti *et al*. 1993, 2004; Grillet *et al*. 1998). The limnological variables in the breeding sites showed stability during the survey, indicating a profile for each type of breeding site. In general, they were within the standards established by CONAMA's environmental resolution for maintaining aquatic life (Brasil 2005).

In addition to limnological variables, climatic factors, rainfall, seasonality, and forest cover (Lima *et al.* 2021; Arcos *et al.* 2021), directly and indirectly influence the co-occurrence of *Anopheles* in breeding sites. A study carried out in fish farming tanks, ponds, and streams in the state of Amapá (Brazilian Amazon), identified eleven limnological variables affecting the distribution of three species of anophelines. Temperature and dissolved oxygen directly affected the presence of *An. triannulatus*, and this relationship was also observed for the dissolved oxygen variable in the present study in the city of Manaus. *An. nuneztovari* was affected by pH, dissolved oxygen, phosphate, turbidity and color in the state of Amapá breeding sites. This same species was also influenced by nutrients present in the water (phosphate and nitrate) at the breeding sites in Manaus (Fig. 4). And the variables pH, total dissolved solids, electrical conductivity, and nitrate contributed to the presence of the malaria vector *An. darlingi* in the Amazon (Barbosa and Scarpassa 2023).

These environmental variables can be considered environmental filters for the occurrence of anophelines in the Amazon. In addition, vector monitoring is an important tool for controlling potential outbreaks of mosquito-borne diseases in the region, such as malaria, in addition to arboviruses such dengue, zika, chikungunya. Therefore, man-made environmental modifications can favor the emergence of pathogens that cause diseases in animals and humans. In addition, the forest around these breeding sites plays an important role in the Culicidae community and in the quality of the aquatic habitat.

Conclusion

The limnological variables explained the presence of some species of *Anopheles* in the breeding sites, and these new habitats classified as artificial favor the development all year round. In addition, the structure of the breeding site, the presence of aquatic macrophytes, water quality, and rainfall are also factors that shape the composition of the community of anophelines and other macroinvertebrates. These new breeding sites help maintain the malaria vector in the region, which is why vector monitoring in these habitats is necessary.

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