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Biting midges (Diptera: Ceratopogonidae) from an urban forest fragment in Central Amazon (Brazil): Effects of opening areas on abundance, richness, and composition

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ABSTRACT

We assessed the immature stages of Ceratopogonidae (Diptera) in artificial containers in an urban forest fragment in Manaus (Brazil), including their behavioral, biological and ecological information. In addition, we evaluated the effects of deforestation in an open and forested area on Ceratopogonidae communities. Immatures were sampled between August 2012 and July 2014 in artificial containers installed in both habitat types. We collected 685 immatures of seven morpho-species of *Bezzia* Kieffer, *Culicoides* Latreille, *Dasyhelea* Kieffer, *Forcipomyia* Meigen, and *Palpomyia* Meigen. In the open area, we recorded higher temperature and electrical conductivity values than in the forested area; however, these variables did not differ between seasons. Water volume was higher in open area and in rainy season, while pH was similar in both areas and seasons. Species richness was higher in forested area, but did not differ between seasons. We did not record differences in abundance between areas or seasons. Community composition differed between areas, but not between seasons. We provide the first records of *Culicoides* (*Hoffmania*) *insignis* Lutz and *C. (Haematomyidium) quasiparaensis* Clastrier in artificial containers from the state of Amazonas. Our results suggest that the preservation of forested areas in Amazonas is fundamental for the maintenance of the life cycle of some species of Ceratopogonidae.

Key words: Amazonia, aquatic insects, artificial containers, deforestation.

INTRODUCTION

Biting midges (Culicomorpha; Ceratopogonidae) are one of the most common and diverse families of Diptera. They are popularly known as “polvorines”,

“manta blanca”, “jejenes”, or “chaquistes” in Spanish-speaking countries, as biting midges, no-see-ums, or punkies in English-speaking countries, and as “maruim”, “mosquito-pólvora”, or “meruim” in Brazil (Spinelli and Wirth 1993). Currently, there are four extant subfamilies, 111 genera, and 6,267 species worldwide (Borkent 2016). The

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subfamilies Leptoconopinae, Forcipomyiinae, Dasyheleinae and Ceratopogoninae include in 52 genera and nearly 1,225 species are known in or have been described from the Neotropical region. In Brazil, 483 species and 31 genera, and in the state of Amazon, 264 species and 28 genera are currently recognized (Santarém and Felipe-Bauer 2016).

These small flies can be vectors of several diseases such as mansonellosis and oncocercosis caused by nematodes, equine encephalitis and other viral diseases transmitted to humans and other vertebrates (Linley et al. 1983, Borkent 2004, Purse et al. 2015). *Culicoides (Hoffmania) insignis* Lutz is a vector of Bluetongue virus (BTV) of domestic and wild ruminants in the Neotropics (Tanya et al. 1992). *Culicoides (Haematomyidium) paraensis* (Goeldi) is incriminated as a vector of oropouche (ORO) virus (Bastos et al. 2012). In Brazil, ORO is the etiological agent of the second most frequent cause of febrile arboviroses (Bastos et al. 2012). Moreover, species in the genera *Atrichopogon* Kieffer and *Forcipomyia* Meigen are ectoparasites of adult and immature insects, for example Lepidoptera and Odonata (Kawahara et al. 2006, Koptur et al. 2013, Nielsen et al. 2014).

The density of Ceratopogonidae larvae can be negatively affected by land use. In general, higher larval abundance and richness have been recorded in forested areas compared to deforested and non-forested areas (Ngai et al. 2008). Moreover, the increase of distance of deforested areas habitats from forest areas also negatively affects Ceratopogonidae fauna (Zimmer et al. 2014). In non-forested and deforested areas, habitats are generally more exposed and may inhibit oviposition of most species (Yanoviak 1999a, 2001). However, some species of Ceratopogonidae have been recorded at different urbanization levels and may occur in higher abundance in some anthropogenic areas (Rubio et al. 2013, Silva and Carvalho 2013).

Ceratopogonidae species are also influenced by intensity and frequency of rainfall and periods of drought (Yee and Juliano 2012, Ptatscheck and Traunspurger 2015). Moreover, communities present in phytotelmata or artificial containers must be adapted to successive evaporation processes (Kitching 2001). Biotic (e.g., predators) and abiotic factors (electrical conductivity, oxygen concentration, water temperature, and pH) of microhabitats may also influence Ceratopogonidae fauna (Cribb et al. 2003, Yanoviak 1999a). For example, a positive relationship between water volume (or containers size) or detritus availability (food) with abundance and richness of this family has been reported (Yanoviak 1999a, b, Gossner et al. 2016).

Ceratopogonidae oviposition can occur in different natural or artificial semi-aquatic and aquatic environments. Their immature stages may occur in diverse habitats in terrestrial (e.g., on wet wood) and aquatic environments (e.g., lakes, rivers, filamentous algae, phytotelmata, and brackish water). Some species are semi-aquatic and can be collected from different substrates (e.g., algae, woods, rocks, decaying fruit), animal footprints, or plants with high moisture contents (e.g., cacti and banana trees, Mercer et al. 2003).

In Central Amazon, taxonomic studies observed Ceratopogonidae development in the laboratory, and described or redescribed species and their life cycle (Ferreira-Keppler et al. 2014). In other taxonomic studies, immatures were sampled in different habitats, for example phytotelmata (Spinelli et al. 2007), macrophytes (Cazorla et al. 2012, Torreias et al. 2013, Díaz et al. 2014), organic polluted streams (Ronderos et al. 2008), and artificial containers in urban areas (Díaz et al. 2013, Ronderos et al. 2013), that analyzed the following genera: *Bezzia* Kieffer, *Culicoides* Latreille, *Dasyhelea* Kieffer, *Forcipomyia*, *Stilobezzia* Kieffer. We studied immatures of Ceratopogonidae in artificial containers in an urban forest fragment

in Manaus (Brazil) that focused on behavioral, biological, and ecological information. In addition, we evaluated the effects of deforestation (opened and closed areas) on Ceratopogonidae communities. We hypothesized higher abundance and richness in closed areas in relation to open areas, mainly due to the higher availability of detritus and better protection for ovipositing females.

MATERIALS AND METHODS

STUDY AREA

We sampled for Ceratopogonidae immatures in a forest fragment near the Instituto Nacional de Pesquisas da Amazônia (INPA), located in the urban area of Manaus (Brazil). The climate is tropical equatorial humid with rainy (November to May) and dry (June to October) seasons. Mean annual rainfall and mean humidity is 2,286 mm³ and 80%, respectively, with a mean annual temperature of 26°C (min = 23°C, max = 31°C, Costa et al. 2013).

SAMPLING PROCEDURE

Ceratopogonidae immatures were sampled monthly between August 2012 and July 2014 in two areas (open and closed) in an urban forest fragment. The open area (03°05'40.4"S; 59°59'22.3"W) has buildings and lacks a vegetation cover; therefore, we installed artificial containers (plastic boxes, Styrofoam boxes, and bamboo internodes) containing water and organic matter (leaves and sticks). The closed area (03°05'44.3"S; 59°59'21.9"W) is a secondary forest with closed vegetation cover and several exotic species (e.g., *Artocarpus heterophyllus* Lam. [Moraceae], *Mangifera indica* L. [Anacardiaceae], *Musa* sp. [Musaceae], *Phenakospermum guyannense* [Strelitziaceae] and *Caladium* sp. [Araceae]). We installed artificial containers (plastic boxes, Styrofoam boxes, cut bamboo, aluminum buckets, toilets, asbestos tanks, and tires) in the area. We used pipettes and plastic trays to collect immatures,

which were transported to the laboratory in 80-mL plastic bottles.

In the laboratory, the immatures were placed in individual plastic containers (2.5 x 2.5 cm) and kept at a constant temperature (~24°C). Every other day, we vacuumed the containers and replenished water and organic matter. Organic matter was obtained from bryophytes to avoid fungal proliferation (Ferreira and Rafael 2006). After emergence, exuviae of adults, larvae, and pupae were preserved in 70% ethanol. For identification, larvae, pupae and adults then dissected and slide-mounted in Canada Balsam (Borkent and Spinelli 2007). Examined, identified specimens were deposited in the Invertebrate Collection of INPA and the Collection of the Entomology Division of the Museu de La Plata (Argentina).

ABIOTIC VARIABLES

Water temperature, pH, and electrical conductivity were measured monthly using portable equipment (waterproof pH meter, conductivity meter, and thermometer; Oakton Instruments, Vernon Hills, Illinois, USA). Water volume was measured with a graduated cylinder (mm).

STATISTICAL ANALYSIS

To evaluate differences in abiotic variables between areas (open and closed) and seasons (rainy and dry), we used Repeated Measure Analysis of Variance (RM-ANOVA). This analysis was also used to test differences of abundance and richness of Ceratopogonidae between areas and seasons. Non-metric multidimensional scaling (NMDS; Sørensen distance) was used to verify the separation of invertebrate communities in relation to areas and seasons. Posteriorly, we tested the significant differences of NMDS groups using Multivariate Analysis of Variance (MANOVA). To perform MANOVA, we used abundance data and Sørensen distance. Similarity percentage analysis

(SIMPER, Clarke 1993) was used to determine the percentage of dissimilarity between areas and seasons. Moreover, SIMPER was used to determine the taxa that most contributed to these differences in invertebrate communities. We used Bray-Curtis similarity matrix and non-transformed data to perform SIMPER.

We modeled the relationship between abundance, richness, and composition (NMDS axis 1) and independent variables (pH, water temperature, electrical conductivity, and water volume) using multiple regression (backward elimination). We used Linear regression (normal distribution) for abundance and composition (continuous data) and a generalized linear model (GLM, Poisson distribution) for richness (count data). Prior to analysis, we assessed the multicollinearity ($r > 0.7$) among explanatory variables by pair-wise correlation analyses. Model selection was done using Akaike's Information Criterion. All statistical analyses were performed in program R (R Core Team 2014), using the package "vegan" (Oksanen et al. 2017).

RESULTS

ABIOTIC VARIABLES

During the experiment, mostly in the rainy season, we recorded a high quantity of plant organic matter (leaves, small fruits, sticks and inflorescences) from the allochthonous vegetation in the open area. Mean pH was similar between areas ($F_{1,17} = 0.70$, $p = 0.414$) and seasons ($F_{1,17} = 3.25$, $p = 0.293$, Table I). Mean water volume was higher in containers located in the open area ($F_{1,17} = 15.10$, $p = 0.001$) and in the rainy season ($F_{1,17} = 63.45$, $p < 0.001$). Water temperature varied between 25.6 and 29°C, with higher values in the open area ($F_{1,17} = 16.87$, $p = 0.001$); however, temperature was similar between the studied seasons ($F_{1,17} = 0.49$, $p = 0.492$). We recorded a significant interaction effect between area and seasons for electrical conductivity ($F_{1,17} =$

12.26, $p = 0.003$). In the closed area, conductivity values were slightly lower in the rainy than in the dry season. On the other hand, in the open area, conductivity increased in the dry season.

FAUNA

We collected 685 immatures from seven species morphotypes (Appendix 1). Immatures were kept in the laboratory and 385 reached the adult stage (open area = 78; closed area = 307). In the open area, Ceratopogonidae were associated with Culicidae (*Aedes aegypti* Linn., *Aedes albopictus* Skuse and *Toxorhynchites* Theobald) Ephydriidae, Psychodidae, Phoridae, Oligochaeta, and Copepoda. In the closed area, we recorded Ceratopogonidae associated with Culicidae, Scatopsidae, Phoridae, and Cecidomyiidae.

Forcipomyia sp., *Culicoides* (*Haematomyidium*) *crucifer* Clastrier and *Dasyhelea eloyi* Díaz & Ronderos were recorded in both areas and seasons (Appendix 1). We recorded *Culicoides insignis* only in the open area during the rainy season. *Culicoides quasiparensis* was recorded only in the rainy season in both areas, while *Bezzia* sp. and *Palpomyia* sp. were found only in the closed area during both seasons (Appendix 1).

BIONOMIC COMMENTS

Larvae of *Bezzia* sp. are elongated with a thin, prognathous cephalic capsule; coloration is slightly yellowish with dark spots visible through their transparent cuticles. They are predators and are known to feed on larvae of Culicidae and Chironomidae (Mullen and Hribar 1988) as well as larvae of some Ceratopogonidae (Grogan and Messersmith 1976). Their locomotion is serpentine, varying from extremely rapid in most aquatic species to extremely slow in others, are phototropic positive and were collected in the deepest parts of the containers within the organic matter. Pupae are dark brown, elongated with pointed terminal

TABLE I
Results of Repeated Measure Analysis of Variance (RM-ANOVA) to test differences in abiotic variables between studied seasons and area; minimum (Min), maximum (Max), mean, and standard deviation (SD) values of abiotic variables in artificial containers in an urban forest fragment in Central Amazon.

Water abiotic variable	Area	Seasons	Min - Max	Mean \pm SD	RM-ANOVA					
					Seasons		Area		Seasons * Area	
					$F_{1,17}$	p	$F_{1,17}$	p	$F_{1,17}$	p
pH	Open	Rainy	7.25 – 8.17	7.73 \pm 0.31	3.25	0.293	0.70	0.414	1.23	0.282
		Dry	6.57 – 8.50	7.67 \pm 0.58						
	Closed	Rainy	7.67 – 8.21	8.03 \pm 0.17						
		Dry	7.23 – 8.26	7.72 \pm 0.40						
Volume (ml)	Open	Rainy	433.33 – 600.00	523.33 \pm 59.81	63.45	< 0.001	15.10	0.001	0.48	0.496
		Dry	200.00 – 500.00	316.25 \pm 97.60						
	Closed	Rainy	358.33 – 528.57	426.08 \pm 62.22						
		Dry	176.67 – 344.29	249.55 \pm 61.15						
Temperature (°C)	Open	Rainy	26.75 – 29.00	27.47 \pm 0.70	0.49	0.492	16.87	0.001	0.06	0.813
		Dry	26.00 – 28.30	27.60 \pm 0.77						
	Closed	Rainy	25.57 – 27.60	26.75 \pm 0.57						
		Dry	26.29 – 27.50	26.96 \pm 0.44						
Electrical conductivity (μ S/m)	Open	Rainy	207.14 – 278.33	232.51 \pm 23.34	1.59	0.225	18.66	< 0.001	12.26	0.003
		Dry	228.57 – 356.67	286.43 \pm 53.21						
	Closed	Rainy	216.67 – 380.00	297.58 \pm 47.91						
		Dry	230.00 – 366.67	288.33 \pm 44.93						

processes. Pupae exhibit circular movements and were collected in the water slide. During adult emergence, pupae are free or adhere to vegetation and other substrates. The development time of 4th instar larvae to pupae was about 15 days, and, from pupa to adult was two to three days.

Larvae of *C. crucifer* are yellowish. They swim rapidly and exhibited greater activity when placed under incandescent light. Larvae swam serpent-like within organic substrate where they remained before pupation. Pupae were also yellowish and occurred alongside coarse particles of debris; they could be collected from margins of aquatic habitats. The development time of 4th instar larvae to pupae was about 25 days, and, from pupa to adult was two to three days.

Larvae of *D. eloyi* were actively creeping and moved towards the bottoms and sides of the

breeding grounds, which are usually shallow (~3 cm). They fed on loose or aggregated, coiled to organic fragments deposited in the bottom of the breeding places next to aquatic larvae of Psychodidae, Ephydriidae, and Phoridae. They exhibited a hypognathous cephalic capsule, pale brown in color and grayish at the dorsal part, with spots along the segments of the thorax and abdomen. At the last instar and in the pupa stage, they had a pale yellow coloration. The development time of the larvae (4th instar) to pupae was about 17 days and that from pupa to adult was one to three days.

Larvae of *Forcipomyia (Euprojoannisia)* sp. had a yellowish coloration. They exhibited slow movements and could be found on the water slide; in some cases, they were dragging themselves along the wall of the nursery or along leaves and

debris. The pupa as yellowish in color. During pupation, the exudation of the larva was retained to the last segments of the abdomen of the pupa. The cephalothorax as darker than the abdomen; segment 9 with elongated and pointed process. Pupae had little mobility, adhering to the side of the nursery often close to the water table until adult emergence. The development time of the larvae (4th instar) to pupae was five days and that from pupa to adult was two to three days.

Larvae of *Palpomyia* sp. were collected near the submerged leaf litter. These larvae were yellowish in color, with darker longitudinal bands visible through transparency. The cephalic capsule was elongated, light brown in color and the caudal segment with wide setae. Larvae swam rapidly, with fast serpentine wave movements. They are predators of small larvae of Culicidae, Chironomidae and other Ceratopogoninae that cohabit these environments. Pupae were dark brown in color, with an elongate, pointed terminal processes. They were found along water margins or adhered to substrates and exhibited lateral movements of their abdomens. The development time of 4th instar larvae to pupations, was about 20 days, and from pupa to adult in 2–3 days.

FAUNA × ABIOTIC VARIABLES

In the open area, we recorded Ceratopogonidae during only 9 (37.5%) of 24 studied months; however, during the dry season of 2013, no individuals were collected (Figure 1). Conversely, we collected immatures during 18 months (75%) in the closed area. *Forcipomyia* sp. and *D. eloyi* were the most abundant taxa. *Forcipomyia* sp. was recorded in higher abundance during the dry season, while *D. eloyi* occurred in higher abundance during the rainy season (Appendix 1). Mean abundance was similar between seasons (rainy = 3.07 ± 5.35 , dry = 1.85 ± 3.73 , $F_{1,22} = 0.74$, $p = 0.398$) and areas (open = 1.51 ± 3.70 , closed = 3.40 ± 5.26 , $F_{1,22} =$

2.44 , $p = 0.132$). The interaction effect between area and seasons on abundance was not significant ($F_{1,22} = 0.80$, $p = 0.380$). Ceratopogonidae richness was high in the closed area (2.00 ± 1.38) in relation to the open area (0.75 ± 1.03 , $F_{1,22} = 24.03$, $p < 0.001$); however, richness was similar between seasons (rainy = 1.50 ± 1.25 , dry = 1.25 ± 1.48 , $F_{1,22} = 0.33$, $p = 0.573$). The interaction effect between area and seasons on richness was not significant ($F_{1,22} = 0.11$, $p = 0.747$).

According to NMDS, it was possible to separate the Ceratopogonidae community into open and closed areas along the first axis; however, there were no differences between the two seasons (Figure 2). Community composition differed between areas (MANOVA: $F_{1,27} = 7.58$, $p = 0.001$), but not between studied seasons (MANOVA: $F_{1,27} = 3.08$, $p = 0.155$). The dissimilarity between open and closed areas was 82.31%. *Forcipomyia* sp., and *Culicoides crucifer*, *D. eloyi*, and *Bezzia* sp. (Table II) were the taxa that most contributed to this dissimilarity.

The selected multiple regression model for abundance explained 8% of the data variation. Ceratopogonidae abundance was negatively related to container water volume (Table III). For richness and composition, the selected multiple regression models explained 29 and 36% of the variation, respectively. Both metrics were positively related to electrical conductivity and negatively to water temperature (Table III).

DISCUSSION

Forcipomyia (Euprojoannisia) is a cosmopolitan subgenus with five records for Brazil in the states of Rio de Janeiro, Bahia, Santa Catarina, and São Paulo (Borkent and Spinelli 2007). Their larvae are semi-aquatic and have been found in floating vegetation, plant concavities (phytotelmata), tree holes, mosses, rock algae, dams with shallow marshes, mud, river margins, macrophytes, and

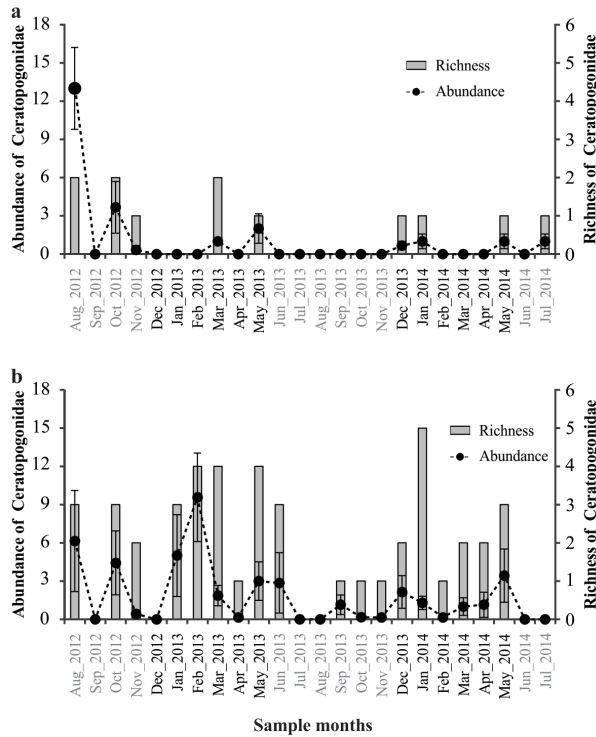


Figure 1 - Mean abundance and richness of Ceratopogonidae sampled between August 2012 and July 2014 in artificial containers located in (A) open area and (B) closed area in an urban forest fragment in Central Amazon. Sample months in black = rainy season; Sample months in gray = dry season.

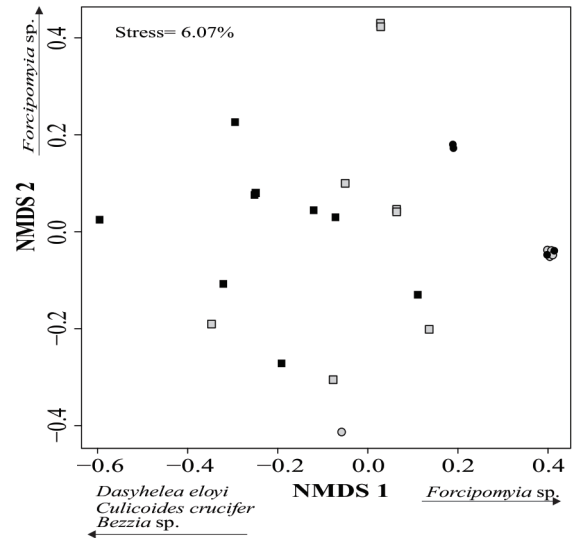


Figure 2 - Non-Metric Multidimensional Scaling (NMDS) and taxa of Ceratopogonidae selected by Similarity percentage tests (SIMPER) to explain the dissimilarity between open and closed areas in terms of Ceratopogonidae abundances in artificial containers located in an urban forest fragment in Central Amazon. Gray square = open area in rainy season; Black square = open area in dry season; Gray circle = closed area in rainy season; Black circle = closed area in dry season.

TABLE II
Mean abundance (SD) and results of the Similarity Percentage Tests (SIMPER) indicating the contribution of Ceratopogonidae taxa to the dissimilarity between open and closed areas in an urban forest fragment in Central Amazon. In bold, values of dissimilarity (%) between groups. Standard deviation values are given in parentheses.

Taxa	Mean abundance		Contribution to dissimilarity (%)
	Open	Closed	82.30
<i>Forcipomyia</i> sp.	1.04 (2.88)	0.58 (1.90)	27.03
<i>Culicoides crucifer</i>	0.29 (0.57)	0.64 (1.03)	21.74
<i>Dasyhelea eloyi</i>	0.13 (0.43)	0.94 (2.09)	15.91
<i>Bezzia</i> sp.	0.00 (0.00)	0.77 (1.94)	11.50

TABLE III
Results of the multiple regression modeling the abundance, richness, and composition (Non-Metric Multidimensional Scaling – Axis 1) of Ceratopogonidae from an urban forest fragment in Central Amazon. In bold, significant *p*-values.

	Abundance			Richness			Composition		
	Coefficients	t value	<i>p</i>	Coefficients	t value	<i>p</i>	Coefficients	t value	<i>p</i>
Intercept	4.627	3.191	0.003	10.037	2.33	0.061	3.914	2.42	0.023
Volume (ml)	-0.007	-2.056	0.047	-	-	-	-	-	-
Temperature (°C)	-	-	-	-0.421	-2.214	0.027	-0.151	2.168	0.040
Electrical conductivity (µS/m)	-	-	-	0.006	2.416	0.016	0.002	2.510	0.019

epiphytic bromeliads (Winder and Silva 1972, Winder 1977, Debenham and Wirth 1984, Torreias et al. 2013). We recorded a higher abundance of larvae of *Forcipomyia* sp. in containers in the open area. Larvae of this genus utilize algae as an important food source (Yeh and Chuang 1996, Wen et al. 2011, Chen et al. 2016). Containers in the open area received a higher incidence of light, which can stimulate algae growth in debris, thereby providing more available food for species of *Forcipomyia*. However, in rainforest areas with low density, primary productivity in aquatic systems is much lower (Vannote et al. 1980).

We recorded a greater abundance of *C. crucifer* and *D. eloyi* in the closed area. In previous studies in tropical regions, larvae of *D. eloyi* were associated with leaves of aquatic macrophytes (Díaz et al. 2013, Torreias et al. 2013). *Culicoides* larvae occur in a wide variety of aquatic and semi-aquatic habitats and feed on debris with abundant organic matter (Silva and Carvalho 2013). Thus, the higher abundance of these two species in the closed area may be associated with higher inputs of detritus (mainly leaves) in the containers. In taxonomic studies of *D. eloyi* and *C. crucifer* with Amazonian material, both species were recorded in artificial containers only in closed areas (Díaz et al. 2013, Ronderos et al. 2013).

The larvae of *C. insignis* were recorded only in the open area during a single sampling event in the rainy season. In Brazil, this species has previously been found in street ditches with still water, small water ponds, and holes dug by crustaceans, *Oedipleura cordata* (Forattini et al. 1956). For adults of *C. insignis*, higher abundance was recorded in anthropic environments (forest edges) compared to forested areas (Veggiani Aybar et al. 2011). The abundance of adults of *C. insignis* is positively correlated with high temperature, precipitation, and humidity (Veggiani Aybar et al. 2012).

Immatures of *Bezzia* sp. and *Palpomyia* sp. were recorded only in the closed area. The larvae of these genera are predators (Mullen and Hribar 1988) and feed on other insects (e.g., Chironomidae and Ephydriidae, Collins 1975) or other invertebrates (e.g. Copepoda, Ronderos et al. 2004). Thus, the presence of *Bezzia* sp. and *Palpomyia* sp. may be related to the greater abundance of non-Ceratopogonidae invertebrates in containers in the closed area as well as the presence of Ephydriidae and Copepoda only in this area.

We recorded higher richness in the closed area compared to the open area; however, abundance was similar in both areas. In general, higher invertebrate richness and abundance are commonly observed in forested rather than non-forested areas, and these areas also differ in species composition (Blaustein et al. 1999, Ngai et al. 2008, Seger et al. 2012). These differences can be attributed mainly to the greater input of detritus in containers in the closed area (Kling et al. 2007, Yee and Juliano 2007). Thus, closed areas provide higher availability of food and habitats for different species, which can stimulate oviposition (Reiskind and Wilson 2004). Moreover, other factors may influence the community of Ceratopogonidae, such as predation or abiotic variables (Seger et al. 2012, Rubio et al. 2013).

We observed a difference in the community of Ceratopogonidae (richness, abundance or composition) between the rainy and the dry season. In general, temperature and precipitation significantly impact insects of phytotelmata and affects their development time as well as the availability of habitats for these organisms (Armbruster et al. 2002, Dézerald et al. 2015, Gossner et al. 2016). However, in our study, water temperature did not differ between the two seasons. In addition, we did not record any dried containers (minimum volume = 176.67 mL), which may have reduced the effects of precipitation on Ceratopogonidae (Rangel et al. 2017). In addition, in the Amazon region, the

higher leaf inputs occur in the dry season (Tonin et al. 2017), which may have attenuated the lower water volume in containers in this season.

In general, the phytotelmata invertebrate fauna is influenced by food availability, water parameters, and seasonality (Torreias and Ferreira-Keppeler 2011, Dézerald et al. 2016). In our study, pH was the only variable that did not significantly affect the Ceratopogonidae fauna. In general, when extreme pH values are not recorded, it is expected that this variable does not strongly affect the community of invertebrates in phytotelmata (Yanoviak 1999b).

In our study, richness and composition were positively associated with electrical conductivity in water containers. Higher values of electrical conductivity result from increased ion concentrations and indicate increased nutrient concentrations in the water (Leite et al. 2016). Thus, higher nutrient values may be associated with the decomposition of allochthonous organic matter (mainly leaves) in containers (Gessner et al. 1999). These higher amounts of nutrients can be used by micro-organisms (e.g., fungi, bacteria, or algae), which are then consumed by invertebrates. Thus, increased food availability results in a more diverse community. In addition, electrical conductivity may be related to the leaching of the detritus (e.g., leaves, branches, and flowers) present in the containers (Yanoviak 1999b).

We recorded a decrease in the abundance of Ceratopogonidae with an increase of the water volume in their containers. In general, there is a positive relationship between invertebrate fauna (abundance and richness) and volume in phytotelmata (Schmidl et al. 2008, Jabiol et al. 2009, Campos 2013, Dézerald et al. 2014). However, water volume has a higher influence on the abundance of invertebrates in environments with frequent droughts, mainly due to egg dehydration and larval mortality (Yanoviak 1999a, Paradise 2004). We did not observe completely dried containers, even in the dry season. In

addition, the number of competitors and predators may be another factor that contributed to the lower abundance of Ceratopogonidae in containers with high water volumes. In previous studies, these organisms have been frequently recorded in higher abundance in larger phytotelmata (Srivastava and Lawton 1998, Yanoviak 1999a).

We observed a negative relation between temperature and Ceratopogonidae richness and composition. Temperature is one of the main abiotic variables that influence the development of immature insects (Spinelli et al. 2005, Bernotiene and Bartkeviciene 2013, Martins et al. 2017). In general, higher temperature values tend to be related to higher abundance of invertebrates (Mayo et al. 2014). However, this increase is not linear and is generally associated with the thermal tolerance limit of each species. In addition, higher temperature values may be associated with lower oxygen values and, consequently, less diverse fauna (Ngai et al. 2008, Fulan et al. 2009).

In conclusion, high conductivity values resulted in high species richness. On the other hand, high water temperature and volume resulted in low richness and abundance levels, respectively. Ceratopogonidae communities did not significantly differ between the two seasons; however, we recorded a higher richness in the closed area. According to our results, the preservation of forested areas is fundamental for the maintenance of the life cycle of some species of Ceratopogonidae.

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Appendix 1

Mean and standard deviation of Ceratopogonidae sampled between August 2012 and July 2014 in artificial containers in an urban forest fragment in Central Amazon.

Month	Seasons	Area	<i>Forcipomyia</i> sp.	<i>Dasyhelea eloyi</i>	<i>Culicoides crucifer</i>	<i>Culicoides quasiparaensis</i>	<i>Culicoides insignis</i>	<i>Bezzia</i> sp.	<i>Palpomyia</i> sp.
AUG/12	Dry	Open	10.33 ± 6.03	2 ± 2.65	2.33 ± 4.04	1 ± 1.73	-	-	-
SEP/12	Dry	Open	-	-	-	-	-	-	-
OCT/12	Dry	Open	-	-	0.33 ± 0.58	-	-	-	-
NOV/12	Dry	Open	-	-	-	-	-	-	-
DEC/12	Rainy	Open	-	-	-	-	-	-	-
JAN/13	Rainy	Open	0.33 ± 0.58	-	-	-	-	-	-
FEB/13	Rainy	Open	0.67 ± 1.15	-	0.33 ± 0.58	-	-	-	-
MAR/13	Rainy	Open	-	-	1.33 ± 2.31	-	0.33 ± 0.58	-	-
APR/13	Rainy	Open	-	-	-	-	-	-	-
MAY/13	Rainy	Open	4 ± 6.93	-	1 ± 1.73	-	-	-	-
JUN/13	Dry	Open	-	-	1 ± 1.73	-	-	-	-
JUL/13	Dry	Open	-	0.33 ± 0.58	-	-	-	-	-
AUG/13	Dry	Open	-	-	-	-	-	-	-
SEP/13	Dry	Open	-	-	-	-	-	-	-
OCT/13	Dry	Open	-	-	-	-	-	-	-
NOV/13	Dry	Open	-	-	-	-	-	-	-
DEC/13	Rainy	Open	-	-	-	-	-	-	-
JAN/14	Rainy	Open	-	-	0.33 ± 0.58	-	-	-	-
FEB/14	Rainy	Open	-	-	-	-	-	-	-
MAR/14	Rainy	Open g	-	0.67 ± 1.15	0.33 ± 0.58	-	-	-	-
APR/14	Rainy	Open g	-	-	-	-	-	-	-
MAY/14	Rainy	Open g	9.67 ± 16.74	-	-	-	-	-	-
JUN/14	Dry	Open	-	-	-	-	-	-	-
JUL/14	Dry	Open g	-	-	-	-	-	-	-
AUG/12	Dry	Closed	7.29 ± 18.41	-	0.71 ± 1.5	0.29 ± 0.76	-	-	-
SEP/12	Dry	Closed	-	-	-	-	-	-	-
OCT/12	Dry	Closed	6.14 ± 13.07	0.29 ± 0.76	0.43 ± 0.79	-	-	-	-

Month	Seasons	Area	<i>Forcipomyia</i> sp.	<i>Dasyhelea</i> <i>eloyi</i>	<i>Culicoides</i> <i>crucifer</i>	<i>Culicoides</i> <i>quasiparaensis</i>	<i>Culicoides</i> <i>insignis</i>	<i>Bezzia</i> sp.	<i>Palpomyia</i> sp.
NOV/12	Dry	Closed	0.57 ± 1.51	0.14 ± 0.38	1.29 ± 3.4	-	-	-	-
DEC/12	Rainy	Closed	-	-	0.29 ± 0.76	-	-	-	-
JAN/13	Rainy	Closed	-	7.29 ± 19.28	-	-	-	4.71 ± 12.03	7.14 ± 18.9
FEB/13	Rainy	Closed	-	7.57 ± 13.19	-	-	-	8.43 ± 18.27	1.86 ± 4.91
MAR/13	Rainy	Closed	-	1.71 ± 4.54	2.71 ± 6.75	-	-	0.71 ± 1.89	0.14 ± 0.38
APR/13	Rainy	Closed	-	1.86 ± 3.76	1.57 ± 3.04	-	-	0.29 ± 0.49	-
MAY/13	Rainy	Closed	-	1 ± 2.65	3.43 ± 9.07	-	-	0.14 ± 0.38	-
JUN/13	Dry	Closed	-	0.14 ± 0.38	0.86 ± 1.46	0.57 ± 1.51	-	2.57 ± 6.8	0.71 ± 1.89
JUL/13	Dry	Closed	-	-	0.57 ± 1.51	-	-	0.39 ± 0.76	-
AUG/13	Dry	Closed	-	-	-	-	-	-	-
SEP/13	Dry	Closed	-	-	3 ± 7.94	0.29 ± 0.76	-	-	-
OCT/13	Dry	Closed	-	0.14 ± 0.38	0.29 ± 0.49	-	-	-	-
NOV/13	Dry	Closed	-	0.14 ± 0.38	0.14 ± 0.38	-	-	-	-
DEC/13	Rainy	Closed	-	-	-	-	-	-	-
JAN/14	Rainy	Closed	-	0.86 ± 1.86	-	-	-	0.29 ± 0.76	-
FEB/14	Rainy	Closed	-	-	-	-	-	0.29 ± 0.76	0.14 ± 0.38
MAR/14	Rainy	Closed	-	0.29 ± 0.76	-	-	-	0.14 ± 0.38	0.14 ± 0.38
APR/14	Rainy	Closed	-	-	-	-	-	0.71 ± 1.89	-
MAY/14	Rainy	Closed	-	-	-	-	-	-	-
JUN/14	Dry	Closed	-	-	-	-	-	-	-
JUL/14	Dry	Closed	-	0.14 ± 0.38	-	-	-	-	-