

ENTOMOLOGY

High mosquito diversity in an Amazonian village of Ecuador, surrounded by a Biological Reserve, using a rapid assessment method

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Abstract

This research represents a study in an Amazonian village that has similar structures to others Kichwa Amazonian villages of Ecuador. We evaluated the diversity, ecology, possibility of mos-

quitoes/pathogens translocation from forest to urban area, and the vulnerability by potential mosquito vectors of diseases through an intensive and fast method done January 2017. Our analyses registered a high diversity of mosquitos in Limoncocha village (33 spp, $H' 2.76$), which includes four new records of species for Ecuador. We propose the biological reserve and the lagoon are determinant environmental factors for the high mosquito diversity, plus the socio-economic characteristics related with a deficient water pipeline supply and lack of solid waste system. Furthermore, the high diversity of sylvan mosquitoes registered throughout the area, that include several potential vectors, suggest a moderate to high vulnerability for the transference of pathogens from the Biological Reserve to the urbanized area, which may increase the circulation of little-known arboviruses (Mayaro, Ilheus, St Louis encephalitis) across Ecuador.

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Introduction

Limoncocha, an Amazonian small village, was founded in 1957 due to the growing interest of oil companies (Texaco and Occidental Oil Gas Corporation, OXY), the Ecuadorian government, and the Summer Institute of Linguistics (SIL) (Ortiz *et al.*, 1995). The SIL mobilized a few young Kichwa people (over 50), from Tena city to borders of Limoncocha lagoon (Konecki *et al.*, 2016). Presently, locality has increased to over 1,500 residents (Instituto Nacional de Estadísticas y Censos, 2010) with poor urban planning that causes deficient water supply, all the while lacking a solid waste disposition, although there are roads systems, dwellings, and educative and health centers with small agriculture lands near the urbanized center. Limoncocha population is influenced by western customs (religion, business, state), hence the lack of social and economic relationship with other ethnic groups in the surrounding area (Burgaleta *et al.*, 2018). This social behavior has created a dependence with the biological resources from the Biological Reserve and Limoncocha Lagoon, which was declared as RAMSAR wetland in 1998 (Ramsar Convention on Wetlands, 1998).

The Biological Reserve presents tropical humid forest and flooded tropical lowland forest, thus, studies in mosquitoes have demonstrated high richness in similar areas (Pecor *et al.*, 2000; Linton *et al.*, 2013; Navarro *et al.*, 2015). Nevertheless, the loss of

primary forests during the last decades in Limoncocha because of human activities could be altering mosquito ecology cycles (Lyimo & Ferguson, 2009; Pongsiri *et al.*, 2009). These changes could have destroyed or created new mosquito breeding sites in this small urban area, hence changing the species richness and abundance following demographic, environmental and social factors (Cardo *et al.*, 2011; Ferraguti *et al.*, 2016). Also, these ecological changes could increase the spread of arboviral disease from the forest to urban areas (Weaver, 2013; Wilder-Smith *et al.*, 2017; Dexheimer Papploski *et al.*, 2018).

In Ecuador, the vector-borne diseases are a big issue in public health. They are mainly transmitted by mosquitoes and cause major outbreaks in tropical and subtropical areas (Navarro *et al.*, 2017). In the Amazonian towns, arboviral diseases as Dengue Virus (DENV), chikungunya Virus (CHIKV), Zika Virus (ZIKV), Yellow Fever Virus (YFV) occur frequently (reports of Dirección Nacional de Vigilancia Epidemiológica) and other probable circulation of viruses are unknown due to the absence of a health network with rapid virus isolation and serology capabilities in little-known arboviruses (Muñoz & Navarro, 2012). This research represents a study in an Amazonian village that has similar structures to other Kichwa Amazonian villages of Ecuador. We evaluated the diversity, ecology, possibility of mosquitoes/pathogens translocation from forest to urban areas, and the vulnerability of potential vector-borne diseases through an intensive and fast method that has been previously proved successful (Barrera *et al.*, 1995; Navarro *et al.*, 2007; Navarro *et al.*, 2015). We hypothesized that Limoncocha, a small Kichwa village with little growth of urban infrastructures, poor pipeline water systems, a weak waste disposal system, and surrounded by a biological reserve with little social and commercial exchange with other communities (including other ethnic groups or localities with a mixed population), must have a diversity of mosquitoes strongly influenced by the mobility of species from the reserve towards the rural-urban population.

Materials and Methods

Study area

The population of Limoncocha is located in the Limoncocha Biological Reserve (RBL) in the Ecuadorian Amazon, Napo Biogeographic Province, and located in the political province of Sucumbíos, Canton Shushufindi (00°24'25"S; 76°37'14"W), parish of Limoncocha, at 203 m of altitude and with a total area of 59,853.32 ha. Its geographical limits are to the north with the parishes of Shushufindi and San Roque, to the south with the province of Orellana, to the east with the parish of Pañacocha, and to the west with the province of Orellana (Figure 1).

The RBL limits to the north with the Blanco River, to the East with the Itaya River, to the south with the Orellana province (Indillama River), to the Northwest with the Yasuni National Park, and to the West with the Jivino River and some populated centers like Playayacu, San Antonio, Limoncocha and Pompella (from north to south). The average annual temperature is 24.9°C, while the average annual rainfall is of 2,965 mm (INAMHi, 2016).

The total population of the parish of Limoncocha consists of approximately 6,700 residents, while in the town there are approximately 1,500 residents of the Kichwa ethnic group, being education, oil and agricultural the population's main activities. Other declared activities are fishing and hunting in the RBL (Konecki *et al.*, 2016). The population consists of approximately 23 housing blocks and 120 buildings. Adjacent to the town is the Napo River (1-1.5 km away) and the Limoncocha Lagoon (abandoned mean-

der of the Napo River, 1,230 years). The RBL is a RAMSAR area (wetland, declared in 1998) and is one of the smallest protected areas in Ecuador (4613 ha). It was declared as a Natural Reserve on September 23, 1985 (Ministerio del Ambiente, 2012).

Mosquito collection

We collected during three consecutive days in an intensive and fast sampling on January 2017 with different trap techniques in the populated zone (64 dwellings or 80% sampled), the ecotone near the lagoon and an intervened area based on a previously proved method (Barrera *et al.*, 1995; Navarro *et al.*, 2007; Navarro *et al.*, 2015). Adult mosquitoes were collected through CDC light traps and human landing catches. The immature specimens were sampled using plastic bomb suckers in different breeding sites, both in natural (Phytotelmata) and artificial containers, and then reared in the laboratory to obtain adult mosquitoes with associated rearing (Belkin, 1965). The genera and species were identified from larvae, females and genitalia males based on *ad hoc* keys (Lane, 1953a, 1953b; Gorham *et al.*, 1967; Valencia, 1973; Zavortink, 1979; Zavortink, 1981; Sallum & Forattini, 1996), as well as contrasting them with the National Collection Reference of Arthropods of Zoonotic importance (CoNRAZ-UCE) and the University collection reference (UISEK). Parallel to biological sampling, a survey about public service quality, socio-economic data, previous outbreaks and vector borne disease symptoms was carried out (Ortega *et al.*, 2018).

Diversity analyses

We quantified the richness and its representativeness (Moreno, 2001) in two habitats (populated zone and ecotone) to obtain the estimated diversity. We evaluated the local diversity (α) using richness (species numbers or S), Shannon-Wiener index (diversity or $H' = -\sum p_i \ln p_i$), and Pielou index (evenness of $J' = H' / \ln S$). We used SHE analysis for studying the relative contribution for richness (S) and evenness (E) in mosquito community diversity (H'), following the relationship: $H' = \ln(S) + \ln(E)$. Finally, we performed a similarity analysis using Jaccard index ($I_j = c / a + b - c$, where: a = species presents in site A, b = species presents in site B, and c = common species in both sites) to show how mosquito composition is related between the two habitats. All calculations were made using PAST software version 2.17 (Hammer *et al.*, 2017).

Results

Mosquito composition

A mosquito survey was carried out in several breeding sites in both urban and ecotone areas. It primarily focused on immature stages with dropper absorber (39% richness collected), as well as CDC light traps (67%) and manual aspirators (6%). Populated zones had the most species (88%) in contrast with ecotone areas (39% near the lagoon and 6% near intervened area). This study identified a total of 291 mosquitoes belonging to 33 species in seven tribes and 12 genera (Table 1). The Culicidae subfamily was the most diverse (94%) and abundant (82%) in relation to the diversity (6%) and abundance (18%) of the Anophelinae subfamily.

The Anophelinae subfamily was collected in adult stages with significant abundance in urbanized and ecotone areas beside the lagoon zone. Within the Culicidae subfamily, the Aedini tribe had three species: *Aedes aegypti* Linnaeus was collected in a barrel in larvae stage and restricted to the urbanized area, while the other two species were collected in adult stages in the ecotone zone. The

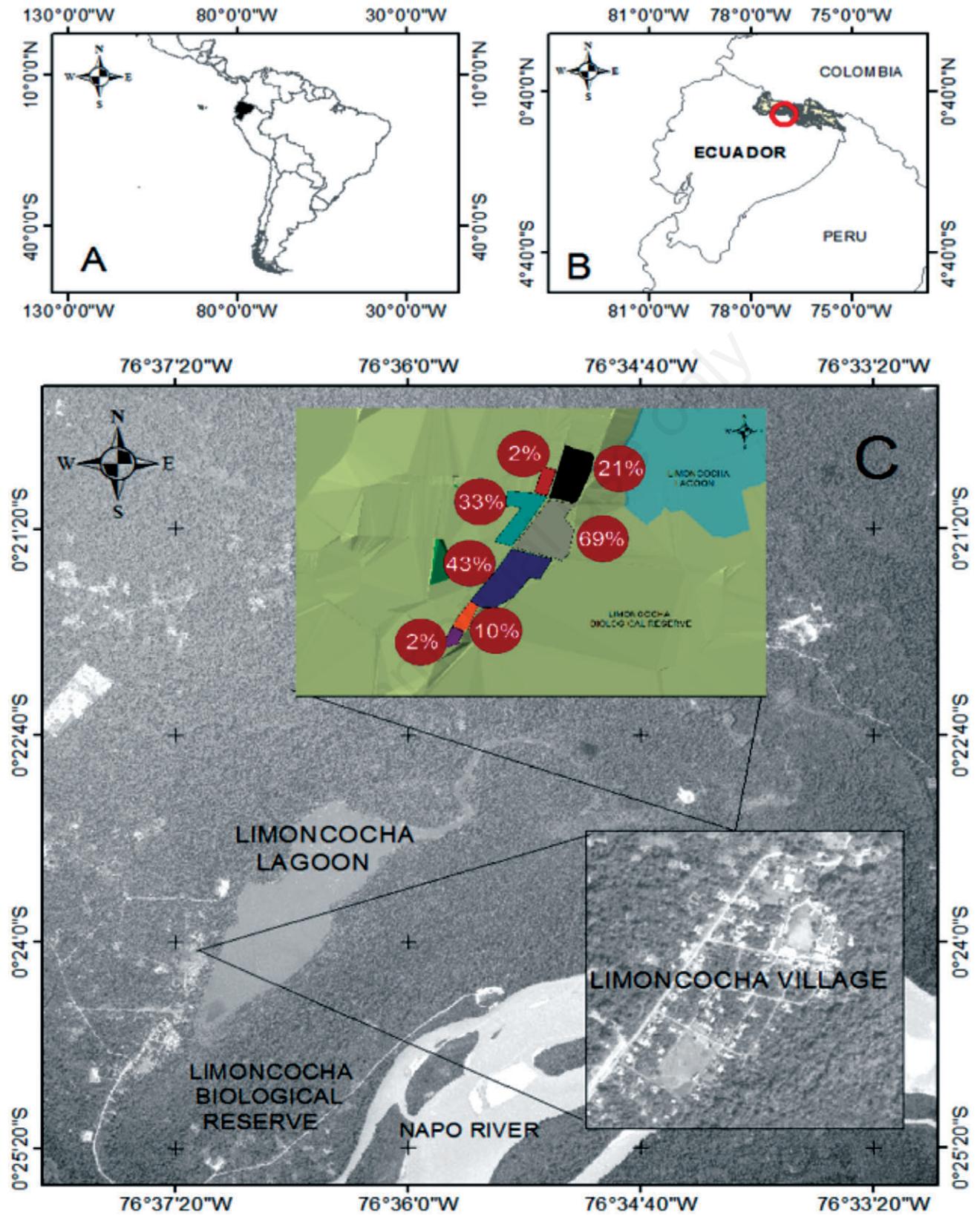


Figure 1. Map of locality. A) Ecuador location in South America, B) Sucumbios Province location in Ecuador, C) Limoncocha Amazonian village with the percent of mosquito diversity by neighborhoods.

Culicini tribe was the most diverse taxa with 14 species in three subgenera: *Culex* (*Carrollia*), *Cx.* (*Culex*) and *Cx.* (*Melanoconion*). The *Cx.* (*Carrollia*) was the only group to be collected in larvae stage and artificial containers, while the *Cx. quinquefasciatus*, another typical urban species, was collected in urban and ecotone areas as well. Mansoniini tribe mosquitoes were collected in adult stages in urban and ecotone areas near the lagoon. Five species were identified in the Sabethini tribe: *Limatus* genus was collected in artificial containers in populated zones; *Wyeomyia melanocephala* Dyar & Knab was found in the leaf axils of phytotelmata plants belonging to the Araceae family (*Xanthosoma* sp and *Colocassia* sp); *Johnbelkinia longipes* Fabricius was registered in urban areas; and *Trichoprosopon compressum* Lutz was collected in broken bamboo internodes. One species of the Toxorhynchitini tribe and four species

of the Uranotaeniini tribe were found in adult stages in urban zones, while *Uranotaenia briseis* Dyar was collected in ecotone areas close to the lagoon (Table 1).

Mosquito richness

The diversity of the whole zone sampled showed high values (H 2.76 in urban and ecotone areas). The richness (S) and equitability (E) were high in the urban zones (S 29 and E 0.82) and low in ecotone zones (S 14 and E 0.78). The diversity indices were complemented with SHE analysis (Figure 2) showing that accumulative values for richness and diversity increased, while evenness values decreased. The mosquito species composition between both sites (urban and ecotone) show a low level of similarity I_j (30%).

Table 1. Species richness by collection site and sampling technique within Limoncocha village.

Species	ENL CDC	ENI CDC	MAs	Urban Area ADr	CDC
<i>Aedes</i> (<i>Ochlerotatus</i>) <i>fulvus</i> Wiedemann [#]		1			
<i>Ae.</i> (<i>Stegomyia</i>) <i>aegypti</i> Linnaeus [#]				1	
<i>Anopheles</i> (<i>Anopheles</i>) <i>apicimacula</i> Dyar & Knab [#]					2
<i>An.</i> (<i>Ano.</i>) <i>nr. mattogrossensis</i> Lutz & Neiva [#]	15		1		33
<i>Coquillettidia</i> (<i>Rhynchotaenia</i>) <i>albicosta</i> Peryassu [*]	3				18
<i>Cq.</i> (<i>Rhy.</i>) <i>juxtamanosonia</i> Chagas	5				6
<i>Culex</i> (<i>Carrollia</i>) <i>bonnei</i> Dyar				15	
<i>Cx.</i> (<i>Car.</i>) <i>infoliatus</i> Bonne-Wepster & Bonne				5	
<i>Cx.</i> (<i>Car.</i>) <i>secundus</i> Bonne-Wepster & Bonne				11	
<i>Cx.</i> (<i>Culex</i>) sp1					1
<i>Cx.</i> (<i>Cux.</i>) sp2					5
<i>Cx.</i> (<i>Cux.</i>) <i>declarator</i> Dyar & Knab [#]				10	
<i>Cx.</i> (<i>Cux.</i>) <i>nigripalpus</i> Theobald [#]	1			3	17
<i>Cx.</i> (<i>Melanoconion</i>) <i>nr. theobaldi</i> Lutz 1904					1
<i>Cx.</i> (<i>Cux.</i>) <i>quinquefasciatus</i> Say [#]	1			1	1
<i>Cx.</i> (<i>Mel.</i>) <i>nr. portesi</i> Senevet & Abonnenc					4
<i>Cx.</i> (<i>Mel.</i>) sp1	1				
<i>Cx.</i> (<i>Mel.</i>) sp3	3				3
<i>Cx.</i> (<i>Mel.</i>) <i>spissipes</i> Theobald [#]	11	4			45
<i>Cx.</i> (<i>Mel.</i>) <i>panocossa</i> Dyar [#]	9				7
<i>Johnbelkinia longipes</i> Fabricius [#]			2	1	
<i>Limatus asulleptus</i> Theobald				2	
<i>Li. durhami</i> Theobald [#]				16	
<i>Mansonia</i> (<i>Mansonia</i>) <i>humeralis</i> Dyar & Knab	1				8
<i>Ma.</i> (<i>Man.</i>) <i>wilsoni</i> Barreto & Coutinho	1				
<i>Psorophora</i> (<i>Grahamia</i>) <i>dimidiata</i> Cerqueira [#]	1				
<i>Toxorhynchites</i> sp. 1				1	
<i>Trichoprosopon compressum</i> Lutz [#]				1	
<i>Uranotaenia</i> (<i>Uranotaenia</i>) <i>briseis</i> Dyar [*]	1				1
<i>Ur.</i> (<i>Ura.</i>) <i>calosomata</i> Dyar & Knab					1
<i>Ur.</i> (<i>Ura.</i>) <i>geometrica</i> Theobald					1
<i>Ur.</i> (<i>Ura.</i>) <i>lowii</i> Theobald					1
<i>Wyeomyia melanocephala</i> Dyar & Knab				8	
Abundance	53	5	3	75	155
Richness	13	2	2	13	18

ENL: Ecotone near to lagoon, ENI: Ecotone near to intervened area, MAs: Manual Aspirator, ADr: Absorber dropper.

^{*}New record for mosquito fauna from Ecuador, [#]species with vector disease role.

Discussion and Conclusions

In tropical zones, vector-borne diseases are associated with social, demographic and environmental factors that primarily affect people who live in rural and urban areas (World Health Organization, 2014). In the Kichwa Amazonian village, we found a high mosquito diversity, including four new species records for Ecuador (Table 1). To understand the high diversity and the potential transmission risk of urban-like arboviruses, it is essential to identify the environmental and social factors that determine the spatial-temporal richness and abundance of the mosquito communities.

It has been reported that ecological changes influenced by human activities allow the expansion and establishment of vectors towards populated areas (Johnson *et al.*, 2008; Pongsiri *et al.*, 2009; Gleiser & Zalazar, 2010; Pires & Gleiser, 2010; Leishnam & Juliano, 2012; Weaver, 2013; Ferraguti *et al.*, 2016; Romero-Alvarez & Escobar, 2017). Only ten sylvan species (30%) have been recorded using artificial/anthropogenic containers (Table 1) due to Kichwa inhabitants using different sources of water supply (wells, rivers, rain), which promotes the use of scarce water storage through large containers (like 200lt drums in the lowland and coastal areas of Ecuador and Latin America) and throughout long-term periods (due the small containers and continuous use). This peculiar storage behavior could explain the low abundance of the typical urban mosquito species reported as vectors with high invasive and vector capacities such as *Aedes aegypti* and *Culex quinquefasciatus* in the populated areas (Gleiser & Zalazar, 2010; Pires & Gleiser, 2010; Stein *et al.*, 2011). The little abundance should be correlated with the Ecuadorian Public Health Ministry epidemiological reports about the absence of recent outbreaks (Dirección Nacional de Vigilancia epidemiológica, 2018).

Mosquitoes exhibit several characteristics concerning the selection of breeding and feeding sites, which can vary spatially and temporally (Lyimo & Ferguson, 2009). However, in relatively stable environments, species find ideal oviposition sites with high

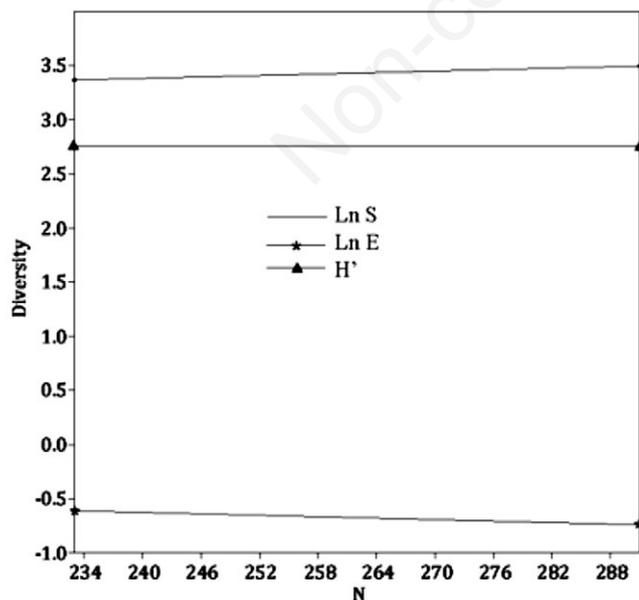


Figure 2. Graph of SHE analysis (S - species, H - Shannon-Wiener diversity index and E - Evenness index) for mosquito assemblages within the Limoncocha village.

survival rates such as floating vegetation in lagoons, as reported for *Mansonia* (Mulieri *et al.*, 2005; Ghosh *et al.*, 2006; Stein *et al.*, 2011), *Uranotaenia*, *Anopheles*, and *Culex* (Lopes & Lozovei, 1995; Stein *et al.*, 2011). We suggest that the Limoncocha lagoon, just behind the village, is the most significant contributing factor to the high richness of mosquitoes belonging to these genera. Although richness was high in urban areas ($H' 2.76$) due to the collection of mosquitoes in adult phases that probably find additional blood-meal resources (humans and domestic animals), their preference for oviposition can be both natural environments and anthropogenic containers available in houses backyards since it has previously been demonstrated in other several mosquito species (Pires & Gleiser, 2010; Cardo *et al.*, 2011; Schmidt *et al.*, 2011; Confalonieri *et al.*, 2012). Our data shows a low similarity of species between the ecotone and urban areas, which are characterized by four abundant species: *Anopheles (Anopheles) nr. matogrossensis* Lutz & Neiva, *Cx. (Melanoconion) spissipes* Theobald, *Cx. (Cux.) nigripalpus* Theobald and *Coquillettidia (Rhynchotaenia) albicosta* Peryassu. These findings suggest that in urbanized areas there is an increase of the vulnerability of the inhabitants towards sylvan transmission by mosquito vectors due to the heterogeneity of ecosystems that occur in the area surrounding the town (lagoon, forests, small crops).

Studies have shown a high diversity of mosquitoes (Culicidae and Anophelinae) in both longitudinal (ecological) and multi-localities sampling with high human population such as Iquitos with 48 spp in 12 months (Johnson *et al.*, 2008), Peru and its vicinity forested areas (40 km around) (96 species in a longitudinal study) (Pecor *et al.*, 2000), Manaus, Brazil with 50 spp in 12 months (Barbosa *et al.*, 2008), Para, Brazil (55 spp, 10 months) (Confalonieri & Costa Neto, 2012), and the Venezuelan Amazonian (Alto Orinoco with 25 mosquito species in 18 Yanomami small and non-westernized villages (Shabonos) (Rubio-Palis *et al.*, 2014). All this data was obtained through the collection of adult mosquitoes using different capture methods. In turn, we identified 33 spp using punctual sampling during three consecutive days of both adult and immature mosquitoes in different types of breeding sites. Such diversity in a punctual sampling like this highlights the need to sample both adult and immature mosquitoes, but also the effectiveness of our three-day sampling, which are ideal for a rapid assessment of biodiversity and epidemiological risks (Navarro *et al.*, 2015; Ortega *et al.*, 2018).

Concerning public service and epidemiological assessment, Schmidt *et al.*, (2011) showed that human populations with high density and better water supply system (about 35,000 residents) have a lower transmission risk than rural and peri-urban area with low populations (3,000 to 7,000) due to poor infrastructure, and absence of water supply and waste storage. However, our case differs from Schmidt *et al.*, (2011) since there is no information about the small Amazonian localities with a low number of inhabitants like Limoncocha. As a result, we are not able to properly evaluate the relationship between the potential vector-borne diversity and the potential risk and vulnerability of arboviral outbreaks.

Limoncocha village belongs to the Amazon, a region with high endemism and biological diversity. However, mosquito richness is still underestimated. This locality is an indigenous Kichwa community with a "Western" housing organization and infrastructure, thus we propose that the biological reserve and the lagoon are determinant environmental sources for the high mosquito diversity. Furthermore, the socio-economic characteristics of the village, including deficient water pipeline and solid waste system, as well as insufficient options to get consumable water due to high and long-term rainfall periods, have brought about a peculiar behavior of water storage and the use of waste containers by its inhabitants.

We suggest that Limoncocha have a low transmission risk of urban-like arbovirus as dengue, Zika and chikungunya due to its low *Aedes aegypti* occurrence. However, the high diversity of sylvan mosquitoes registered throughout the area, including several potential vectors, suggest a moderate to high vulnerability of the potential transference of pathogens from the Biological Reserve (including the lagoon) to the urbanized area, knowing the circulation of little-known arboviruses (Mayaro, Ilheus, St Louis encephalitis) due to the presence of antibodies in soldiers who have lived in the Amazon (Calisher, *et al.*, 1983; Horby, 2007; Manock *et al.*, 2009; Izurieta *et al.*, 2011).

This study will serve as a baseline for comparison with other locations of similar size in northern-south transects of the Ecuadorian Amazon, and also to compare the mosquito diversity with other ecosystems/land use surrounding the Limoncocha village, both researches in progress.

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