

# RIVER BOATS AS A MEANS OF EXPANSION OF *Aedes aegypti* TOWARDS BORDER ZONES OF THE PERUVIAN AMAZON

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## ABSTRACT

**Objectives.** To assess the entomological risk of *Aedes aegypti* in boats traversing border river routes in Loreto. **Materials and Methods.** The study population consisted of mosquitoes present in three boats covering border routes in Loreto. The entomological risk of *Aedes* was determined through ovitraps, the inspection of breeding sites, and the collection and taxonomic identification of adult mosquitoes. **Results.** The entomological risk varied according to the route and the season. A medium to very high entomological risk was identified in the high-water season and on the outward route to the border areas. The predominant vector population in the low-water season was *Mansonia sp.* (74.8%), *Culex sp.* (12.8%), and *Aedes aegypti* (0.4%); in the high-water season, *Culex sp.* (45.1%), *Mansonia sp.* (26.8%), and *Aedes aegypti* (19.7%). In no case did we find *Aedes albopictus*. **Conclusions.** There is moderate to high entomological risk during the high-water season in riverboats traveling from Iquitos to the border areas of Loreto. Our results show that river boats are a means of expansion of *Aedes aegypti*.

**Keywords:** Mosquito vectors; *Aedes*; Sanitary control of harbors and crafts; Border areas (source: MeSH NLM).

## INTRODUCTION

*Aedes aegypti* is the vector responsible for the spread of diseases like dengue, chikungunya, and zika, which are increasingly important worldwide both for their disease burden and for their epidemic potential<sup>(1-3)</sup>. The prevention of these diseases through a vaccine and other innovative interventions is promising, but they are not yet available in the short term<sup>(4,5)</sup>. Therefore, vector surveillance continues to be the main tool for the prevention and control of *Aedes aegypti*<sup>(6)</sup>.

Globally, it has been demonstrated that the expansion of economic activities, such as ground or river transportation, from urban to rural areas, has led to the spread of *Aedes aegypti*, increasing the risk in the rural areas<sup>(7-9)</sup>. Socio-economic determinants, such as poverty and lack of basic services, combined with the vector increase, have made these areas targets for epidemic outbreaks<sup>(7,10)</sup>.

The presence of *Aedes aegypti* and *Aedes albopictus* has been reported in Manaus (Brazil) and Leticia (Colombia)<sup>(7,9)</sup>, and in the region of Loreto (Peru) *Aedes aegypti*<sup>(11,12)</sup> is present in most of its districts. In this epidemiological context, the navigation of river boats could spread *Aedes* from populated cities to remote areas with low vector density<sup>(8,9)</sup>. The increase in the mosquito population in border regions could have serious consequences for the indigenous population; it could cause the collapse of health services and increase morbidity and mortality from dengue, chikungunya, and zika due to the limited response capacity of the health centers in rural areas.

The Executive Directorate of Environmental Health of Loreto (DESA Loreto), which is the local health authority, conducts the entomological surveillance of *Aedes aegypti* in the community, inside and around dwellings. Surveillance is carried out by a health professional, who

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visits and inspects water containers and collects the larvae in them<sup>(11)</sup>. This practice has obstacles such as the scarcity of human resources, limited economic resources for extramural activities, and delays in the collection and processing of the epidemiological information<sup>(13)</sup>. As a result, the use of ovitraps was introduced in 2015, as part of the entomological surveillance of *Aedes aegypti*<sup>(14,15)</sup>.

River boats are a popular means of transportation in areas of the jungle and have characteristics conducive to the reproduction of *Aedes*. They have spaces with shade and humidity, transport water in different containers and move with cargo and passengers through populated and rural cities<sup>(9)</sup>. Therefore, we consider it imperative to know the entomological risk, presence, and abundance of *Aedes* in these vessels and demonstrate that they can be vehicles for the spread of *Aedes* in the Amazon basin. We also consider it necessary to evaluate the use of ovitraps to detect populations of *Aedes aegypti*<sup>(16)</sup> in this new scenario, not described in previous publications.

The objective of our study was to evaluate the entomological risk posed by *Aedes aegypti* during the river travel of boats with routes along Loreto border areas.

## MATERIALS AND METHODS

### DESIGN AND LOCATION

A longitudinal descriptive study was conducted in the Loreto region of the Peruvian Amazon. Loreto is an endemic region and has the highest frequency of cases of dengue, zika and malaria in Peru. The study was carried out from September to November 2016 (high-water season) and from May to July 2017 (low-water season) in river boats with border routes toward Caballococha (3°54'29.44"-S and 70°30'58.02"-W and 77 masl on the banks of the Amazon River); Santa Rosa (4°13'10.41"-S and 69°57'59.64"-W and 100 masl on the banks of the Amazon River, at the border with Colombia and Brazil), and El Estrecho (2°27'1.6"-S and 72°40'4.4"-O and 110 masl on the banks of the Putumayo River).

Three-story vessels carrying passengers and cargo, with an average length of 50 meters, were selected. Thirteen vessels traveling to Caballococha and Santa Rosa and four vessels going to El Estrecho met these criteria. One vessel per route was selected at random.

### DATA COLLECTION

Authorization from the director of DIRESA Loreto (Official Letter No. 483-2015- GRL-DRSL/30.09-INVESTIGACION)

## KEY MESSAGES

**Research motivation.** To know the entomological in situ risk of *Aedes* in river boats with border routes.

**Main Findings.** River boats are a means of dispersion of *Aedes aegypti*. The entomological risk of *Aedes aegypti* was low in low-water season and going back to Iquitos; medium to very high in high-water season and going toward border areas. The vector population found was: *Mansonia sp.*, *Culex sp.*, and *Aedes aegypti* in low-water and *Culex sp.*, *Mansonia sp.*, and *Aedes aegypti* in high-water season. No *Aedes albopictus* was found.

**Implications.** There is a moderate to high entomological risk during the high-water season in river boats traveling from Iquitos to the border areas of Loreto.

for the execution of the project was obtained. The authorizations were signed, which allowed entrance to the vessels, as well as the presence of a member of the research team to travel on the routes and perform entomological surveillance along the way. Two trips were made, one during the high-water season (characterized by the increase in river flow) and one during the low-water season (characterized by the decrease in river flow)<sup>(17)</sup>. Three different vessels were analyzed on their way to Caballococha, Santa Rosa and El Estrecho. The entomological surveillance was carried out during the outward and the return journey, performing a total of two revisions per boat, for the collection of eggs, larvae/pupae, and mosquitoes (Table 1) (Figure 1).

### COLLECTION OF INFORMATION

The information was collected using an instrument designed and digitized on an electronic tablet, using the Open Data Kit with data capturing, stored locally and transferred later on to the cloud (Google Drive) with a backup copy available off-line and transferred later to the Center for Research in Tropical Diseases (CIETROP) of the National Health Institute (INS). The strips of paper with eggs were captured with HAD-CCD cameras of 20.1 megapixels, stored in memories and processed with the program Image J for strips of up to 20 eggs; for a larger number of eggs the Stereomicroscope SZX16 was used. All potential larval and pupal hatches were reviewed. Adult mosquitoes were collected with Prokopack aspirators. The coordinates were taken with a Garmin Etrex 20X GPS.

### ENTOMOLOGICAL RISK ASSESSMENT

Entomological risk was defined as the presence and abundance of insect vectors of a disease in a given place<sup>(18)</sup>.

**Table 1.** Characteristics of the boats according to season and route

Characteristics	High-water			Low-water		
	Caballococha	Santa Rosa	El Estrecho	Caballococha	Santa Rosa	El Estrecho
Departure time	20:41:00	22:43:00	04:00:00	21:10:00	22:10:00	06:50:00
Travel time (round trip) days	4	5	25	5	6	30
Number of passengers	145	143	40	134	111	50
Number of passengers per floor*	P1: 5 P2: 70 P3: 70	P1: 6 P2: 80 P3: 56	P1: 8 P2: 12 P3: 20	P1: 7 P2: 72 P3: 55	P1: 7 P2: 56 P3: 47	P1: 10 P2: 20 P3: 20
Number of passengers per room	2	3	2	2	2	2
Number of containers inspected	23	30	27	14	38	21
Transporting drinking water	No	No	Si	No	No	Si
Number of floors	3	3	3	3	4	3
Number of rooms per floor	P1: 4 P2: 4 P3: 5	P1: 4 P2: 4 P3: 4	P1: 4 P2: 4 P3: 3	P1: 4 P2: 4 P3: 5	P1: 4 P2: 4 P3: 4	P1: 4 P2: 4 P3: 3
Wall material	Metal	Metal	Metal	Metal	Metal	Metal
Roofing material	Wood/metal	Metal	Metal	Madera/metal	Metal	Metal
Floor material	Metal	Metal	Metal	Metal	Metal	Metal
Window material	Metal, glass, and mica	Metal, glass, and mica	Mesh treated for bugs	Metal, glass, and mica	Metal, glass, and mica	Mesh against bugs
Kitchen	Gas	Gas	Gas	Gas	Gas	Gas
Source of cooking water	River	River	Treated	River	River	Treated
Waste disposal	Bags	Bucket	Bucket and bags	Bags	Bags	Bags

P1: first floor, P2: second floor, P3: third floor

It was defined using the following indicators: ovitraps positivity index (OPI), egg density index (EDI)<sup>(14)</sup> and adult index (AI)<sup>(13)</sup>.

Inspection of eggs in ovitraps: ovitraps are plastic deposits that carry an identification label on the outside. Paper towel is used as medium and grass infusion is used as attractant. The criterion used for the placement of ovitraps was based on the description contained in the technical standard for dwellings<sup>(11)</sup>. Each floor of the boat was considered a dwelling and ovitraps were placed every 50 meters, taking into account that the flying range of the mosquito is between 50 and 100 meters; to place the ovitraps inside the boats, dark areas were chosen, free of wind flows and with no exposure to rain<sup>(14)</sup>. When the paper was positive, it was picked up and replaced with another. Then, the image was captured, the geographical location of the place was taken<sup>(19)</sup> (Table 2) and the paper was stored in a labeled plastic bag. Inspection of larvae/pupae: on each of the floors of the boats, containers with water that could contain larvae or pupae were located, in accordance with the format for hatcheries used by DESA Loreto<sup>(11,20)</sup>.

Adult capture: adult mosquitoes were captured with Prokopack aspirators along the walls, floors and spaces, coinciding with their feeding times (8.00 to 10.00 h and 17.00 to 19.00 h)<sup>(21)</sup>.

Later on, they were exposed to ethyl acetate for their preservation and transport in petri dishes until they were taken to the CIETROP laboratory to be identified.

**DATA ANALYSIS**

The entomological risk was measured using entomological indicators such as the *Aedes* density index based on the egg density index (EDI), which is obtained dividing the number of eggs found by the number of positive ovitraps, classifying as low risk: from 1 to <5 eggs, medium risk: 5 to <20 eggs, high risk: 20 to <40 eggs and very high risk: >40 eggs<sup>(14)</sup>. The Adult Index (AI) refers to the number of mosquitoes, classifying as low risk from 1 to 3 mosquitoes and high risk: >3 mosquitoes<sup>(13)</sup>. The *Aedes* positivity index based on the ovitraps positivity index (OPI), which is obtained dividing the number of positive ovitraps by the number of exposed ovitraps x 100, classifies as low risk: <5%, medium

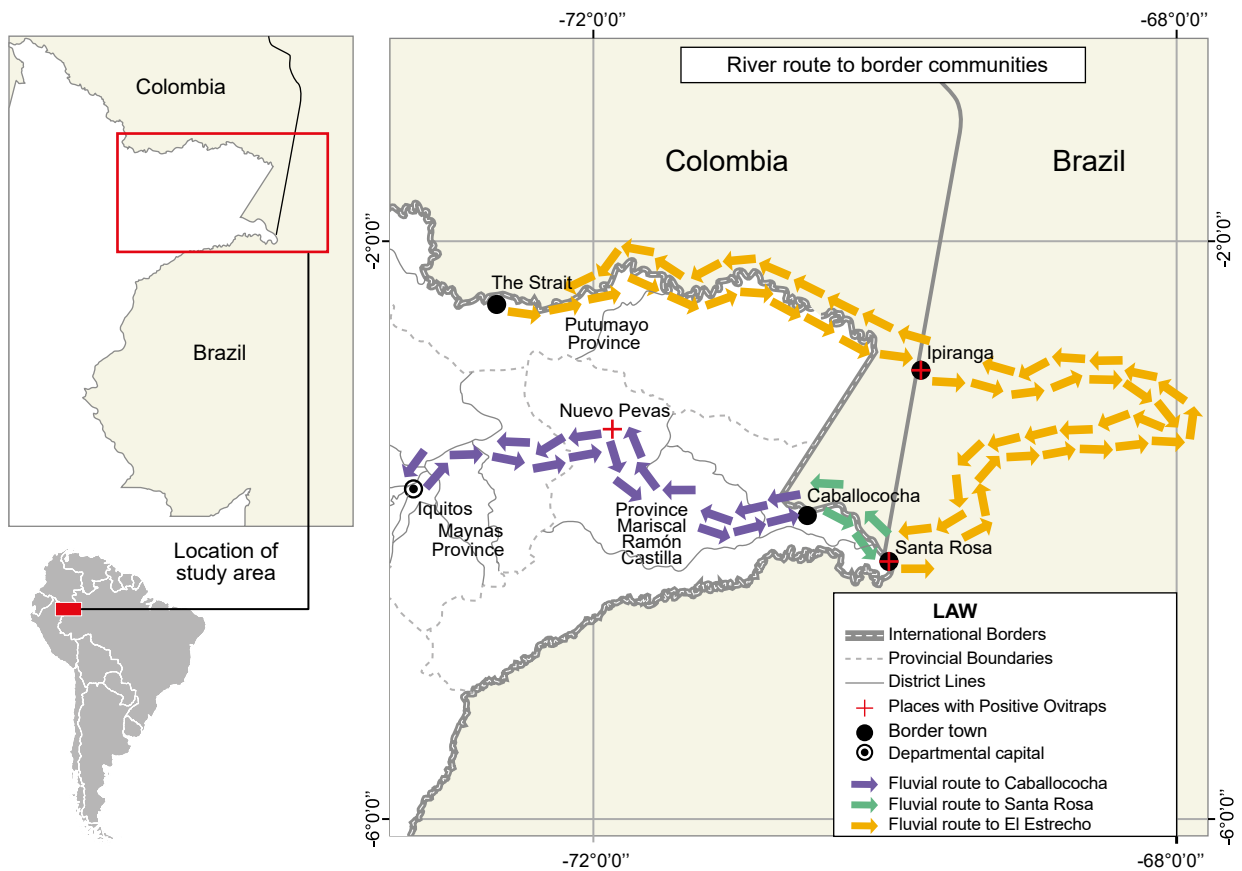


Figure 1. Map of the route followed by the boats towards the border towns (round trip)

risk: 5% to <20%, high risk: 20% to <40% and very high risk: >40% (14). The collected vectorial populations were identified with taxonomic keys (22).

The statistical procedures for data processing were expressed through frequencies and percentages. Version 21 of statistical package SPSS was used.

Table 2. Geospatial location of the ovitraps in river boats

Georeferencing		Localities	Length of stay of the barges	Inhabitants	Positivity of ovitraps
Latitude	Length				
-3.487269	-72.190797	Apayacu	1 h	285	-
-3.322942	-71.862146	Pevas	2 h	3788	+
-3.371125	-71.776023	Nuevo Pevas	30 min	482	-
-3.758134	-71.624055	Cochiquina	2 h	1041	-
-3.806504	-71.573452	San Antonio	1 h	709	-
-3.863192	-71.352887	San Isidro	1 h	525	-
-4.017102	-71.100457	San Pablo	2 h	3137	-
-3.927604	-70.778901	Chimbote	2 h	344	-
-3.905087	-70.517707	Caballococha	2 h	9051	-
-4.219842	-69.955005	Santa Rosa	2 h	970	+
-4.3524	-70.040455	Islandia	1 day	1692	-
-2.148826	-71.753218	Ipiranga	1 h	456	+
-2.893369	-69.734015	Tarapaca	1 h	289	-
-2.539835	-70.429	Huapapa	1 h	355	-
-2.385124	-71.181284	Remanso	1 h	412	-
-2.445288	-72.668377	El Estrecho	1 week	3056	-

**ETHICAL ASPECTS**

This research posed a minimal risk to the people on the participating riverboats. The entomological inspections carried out in the study were identical to the ones carried out by DESA Loreto. There was no increased risk of transmission of these diseases to participants beyond the risk experienced by living in an endemic area. The results of this study will be of great benefit to local vector control activities. All ethical considerations were reviewed and approved by the Animal Ethics Research Committee of the INS through directorate resolution N°580-2016-OGITT-OPE/INS.

**RESULTS**

*DENSITY AND POSITIVITY INDEX OF Aedes sp.*

On the route to Cabalococha, the IDH and IA indices were 0% on the outward and the return trips, both in growing and in emptying seasons. On the route to Santa Rosa during the growing season, 26 to 66 eggs were found, while no eggs were found during the emptying season. On the route to El Estrecho, up to seven eggs were found during the growing season. The interpretations of these findings are detailed in Table 3. In none of the routes was it possible to find eggs of *Aedes albopictus*.

On the route to Cabalococha the OPI was low during the outward leg and the return leg, in both growing and emptying seasons. On the route to Santa Rosa, the OPI was 14% on the round trip during the growing season, and in the emptying season it was 0%. On the route to El Estrecho, the OPI was 20% in both growing and emptying seasons during the outward journey and 0% on the return journey (Table 3). None of the routes showed *Aedes albopictus* in the ovitraps.

The containers that could house *Aedes larvae* and/or pupae were inspected; we found, in greater proportion, both in

growing and in emptying seasons, servers (plates, frying pans, plate holders), buckets and pots and, to a lesser extent, tanks, cylinders and tires. No positive hatchery was found.

*VECTOR POPULATIONS*

The vector population found was composed of *Aedes aegypti*, *Aediomia*, *Anopheles nuneztovari*, *Anopheles peryassui*, *Anopheles trianulatus*, *Coquilletidia*, *Culex melanoconium*, *Culex sp.* and *Mansonia sp.* The predominant vector population in the emptying season was *Mansonia sp.* (74.8%), that transmits lymphatic filariasis, *Culex sp.* (12.8%), that transmits encephalitis; and oropuche, filariasis, Nile virus and *Aedes aegypti* (0.4%), that transmit dengue, zika and chikungunya (23.24); during the growing season, more prevalent were *Culex sp.* (45.1%), *Mansonia sp.* (26.8%) and *Aedes aegypti* (19.7%). In Table 4, the vectorial populations are described according to trajectory and season.

**DISCUSSION**

It was demonstrated that during the growing season an entomological risk of *Aedes aegypti* exists in river boats that travel from endemic urban populations to non-endemic rural areas. We can therefore consider river boats as a means of spreading *Aedes aegypti*.

The main means of transportation in the Amazon are river boats, which have different characteristics depending on the number of passengers, type of cargo and distance to travel. Different locations have proved to be reservoirs of *Aedes aegypti*, however, those studies were carried out in cities, houses or ports, and not on the river route of a vessel<sup>(8,9)</sup>. During the travelling of the fluvial boats no positive breeding places were evident, but potential breeding places were identified, such as servers (plates, frying pans, plate holders), buckets and

**Table 3.** Density index and positivity index of *Aedes* according to season and route

Route	EDI				OPI				AI			
	Growing		Emptying		Growing		Emptying		Growing		Emptying	
	Out	Return	Out	Return	Out	Return	Out	Return	Out	Return	Out	Return
Cabalococha	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Santa Rosa	Very high	High	Low	Low	Med	Med	Low	Low	Low	Low	Low	Low
El Estrecho	Medium	Low	Low	Low	High	Low	High	Low	Med	Low	Low	Low

EDI: egg density index, OPI: ovitraps positivity index, AI: adult index

**Table 4.** Distribution of vectors in fluvial vessels according to season and route

Variable	Caballococha				Santa Rosa				El Estrecho				Total			
	Growing		Emptying		Growing		Emptying		Growing		Emptying		Growing		Emptying	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Mosquito species																
<i>Aedes aegypti</i>	0	0.0	0	0.0	1	8.3	0	0.0	13	40.6	1	7.7	14	19.7	1	0.4
<i>Aediomy</i>	1	3.7	15	37.5	0	0.0	12	5.2	1	3.1	0	0.0	2	2.8	27	9.5
<i>Anopheles nuneztovari</i>	0	0.0	0	0.0	0	0.0	2	0.9	0	0.0	0	0.0	0	0.0	2	0.7
<i>Anopheles peryasui</i>	0	0.0	0	0.0	0	0.0	0	0.0	1	3.1	0	0.0	1	1.4	0	0.0
<i>Anopheles trianulatus</i>	0	0.0	0	0.0	0	0.0	1	0.4	0	0.0	0	0.0	0	0.0	1	0.4
<i>Coquilletidia</i>	0	0.0	0	0.0	0	0.0	3	1.3	2	6.3	0	0.0	2	2.8	3	1.1
<i>Culex melanoconium</i>	0	0.0	0	0.0	1	8.3	1	0.4	0	0.0	0	0.0	1	1.4	1	0.4
<i>Culex sp.</i>	16	59.3	7	17.5	7	58.3	17	7.5	9	28.1	12	92.3	32	45.1	36	12.7
<i>Mansonia sp.</i>	10	37.0	18	45.0	3	25.0	193	84.3	6	18.8	0	0.0	19	26.8	211	74.8
Sex of mosquitoes																
Female	21	77.8	33	82.5	10	83.3	214	93.4	22	68.7	11	84.6	53	74.6	258	91.5
Male	6	22.2	7	17.5	2	16.7	15	6.6	10	31.3	2	15.4	18	25.4	24	8.5
Boat floor																
P1 (floor level)	8	29.6	19	47.5	8	66.6	40	17.5	22	68.8	2	15.3	38	53.5	61	21.6
P2 (2 m high)	6	22.2	8	20.0	2	16.7	65	28.4	5	15.6	5	38.5	13	18.3	78	27.7
P3 (4.5 m high)	13	48.2	13	32.5	2	16.7	124	54.1	5	15.6	6	46.2	20	28.2	143	50.7

P1: first floor, P2: second floor, P3: third floor

pots and, to a lesser extent, tanks, cylinders and tires. In spite of not having found immature forms in the potential hatcheries and a smaller adult population of *Aedes aegypti* with respect to the other vectors, the present study demonstrates that fluvial boats constitute a means of spreading the *Aedes aegypti* to less populated places that have the appropriate conditions for its establishment.

Assessing the entomological risk during the journey made it possible to learn about the operational dynamics of the vessels *in situ* and identify specific targets for future interventions in health. For example, we now know that in the route to the town of El Estrecho, being the longest, the water supply is transported in unprotected containers, and it was on this route that a higher EDI and OPI was evidenced.

During one of the periods of the study (September to November 2016) an outbreak of zika occurred in the city of Iquitos, which spread rapidly to the province of Ramón Castilla and to rural areas of the province of Maynas, areas through which the fluvial boats that were evaluated in our study travelled. Although the design of our research does not allow us to state that river boats dispersed zika, it is possible that they contributed to this epidemic, as described in other epidemics worldwide<sup>(3,25)</sup>.

Our results on *Aedes aegypti* reinforce what has been previously published about its capacity to follow an

expansion pattern linked to the growth of cities, new roads and along rivers<sup>(8)</sup>. The capacity of *Aedes aegypti* to infest growing urban areas can be accelerated by the means of transportation that can transport these vectors to urban areas such as Iquitos, especially if they return from areas that border the Brazilian and Colombian Amazonia, where there is a presence of *Aedes albopictus* and chikungunya outbreaks.

No eggs, pupae or adult forms of *Aedes albopictus* were collected, coinciding with what was reported by a local study in a border region of Loreto<sup>(26)</sup>. Possibly the factors that prevent the establishment of *Aedes albopictus* could be the Amazon River acting as a geographic barrier, and the fact that the products transported on boats from Leticia and Tabatinga to Santa Rosa do not function as breeding grounds for this vector. Likewise, it is not possible to confirm the absence of the vector when the surveillance carried out lasted less than one year<sup>(11)</sup>.

The EDI and AI were higher during the growing season and when the boats departed from Iquitos, an endemic city with high population density<sup>(15,27)</sup>; the population density and rainfall coincide with an increase in the presence of the vector at every stage<sup>(8)</sup>.

In connection to the vector population, *Aedes aegypti* was not predominant and was only greater during growing

season. The mosquito with the highest population was *Mansonia sp.* followed by *Culex*, evidencing the richness of mosquito species and their seasonal distribution. Even though different vectors in adult forms were found during the inspection of ovitraps, only egg from *Aedes*, not from other vectors, was evident <sup>(28)</sup>. The emergence and re-emergence of some mosquito-borne illnesses is one of the most relevant aspects in the American continent, reason why knowing the mosquito fauna, as well as its distribution, is imperative for adequate vector control and for establishing strategies to prevent the spread of communicable diseases <sup>(29)</sup>.

Ovitraps made it possible to determine a very high EDI on one route, demonstrating their usefulness and sensitivity in river transport, which confirms that they can be used in new places and surveillance conditions or research studies <sup>(12,30)</sup>.

Some limitations have to do with the design, which did not allow to assess the degree of parity of mosquitoes, perform molecular tests, and include the evaluation of people with fever during the river trip. Despite these limitations, our study has shown that river boats are a means of expansion of *Aedes aegypti* to rural areas of the Peruvian Amazon. Local health authorities should therefore strengthen vector surveillance in river boats.

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