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POTENTIAL MOSQUITO BREEDING SITES IN EMPTY SHELLS OF THE AFRICAN SNAIL, *LISSACHATINA FULICA* IN CALI, COLOMBIA

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ABSTRACT. Habitat descriptions for vector larvae of public health importance are fundamental to the design of cost-effective control measures. Faced with the invasion of the giant African snail, *Lissachatina fulica*, the objective of the present study was to determine the role of empty giant African snail shells as mosquito breeding sites. Three experiments were performed under field conditions in four microenvironments on the banks of the Lili River in the city of Cali. Additionally, two artificial containers (motorcycle tires and plastic cups) were placed in each microenvironment. In experiments 1 and 2, the empty giant African snail shells of different sizes, which contained a previously determined water volume, were positioned in 4 selected microenvironments. In experiment 3, empty giant African snail shells of different sizes were also located in the four microenvironments during the rainy season, and the water volume in each shell was the result of natural rainfall accumulation. The variables final water volume, shell size, and mosquito density were evaluated in the three experiments, using a total of 92 shells. The dominant mosquito species identified were *Limatus durhamii, Aedes albopictus,* and *Ae. aegpti*. Experiment 1 revealed that giant African snail shells measuring less than 60 mm had limited breeding potential, whereas experiment 3 showed that shells larger than 100 mm had higher numbers of mosquito larvae. There was a significant association between shell size, water volume, and mosquito density. A total of 757 mosquitoes were identified. This is the first exploratory study under field conditions in Colombia to evaluate empty giant African snail shells as breeding sites for mosquitos of public health importance. Results of this study provide new information that should be considered in control strategies in areas with concomitant presence of giant African snails. Entomological findings and their epidemiological importance are discussed.

KEY WORDS Arbovirus, breeding sites, culicidae, giant African snail, mosquitoes

INTRODUCTION

Mosquitoes are considered a threat worldwide because of their ability to transmit the pathogens of interest to human and veterinary health. More than 80% of the global population lives in large cities, where diseases transmitted by mosquitoes cause approximately 700,000 deaths each year (WHO 2017, 2022). Mosquitoes are obligatory hosts of several infectious agents and can adapt to a wide range of ecological conditions. The transmission patterns of arboviral diseases as well as their geographical expansion are related to environmental changes, including urbanization (Gubler 2011, Weaver 2013). Land use changing mainly through agricultural activities and the construction of large urban areas to meet human needs can negatively affect the ecological dynamics of ecosystems and biodiversity (Shochat et al. 2006). The presence and quality of larval breeding sites are among the most important determinants of the abundance and distribution of adult mosquitos. Therefore, understanding the dynamics and productivity of these sites could allow the improvement of in situ control strategies (Rejmankova et al. 2013, Nihad et al. 2022). In urban ecosystems, synanthropic mosquitoes use a wide range of aquatic environments that are associated with housing and public spaces, mainly artificial containers made of plastic, metal, glass, rubber, cement, clay, and ceramics (Zahouli et al. 2017, Alarcón-Elbal et al. 2021). Other natural microhabitats found in cities include tree holes, rock hollows, wells, bromeliads, bamboo stumps, empty snail shells, coconut husks, etc. (Fontenille and Toto 2001). Some changes affecting the biodiversity of species in urban environments respond to 1) the accidental introduction of species through aerial, terrestrial, and maritime transport, and 2) the intentional introduction of species with different purposes such as biological controls, pets, medicinal use, and other human uses (McKinney 2001). The number of generalist species can increase as a consequence, resulting in the loss of native species and the establishment and colonization of opportunist and invasive species (Chaves et al. 2011, Johnson and Munshi-South 2017). An example of biological invasions in South America has been the arrival of the giant African snail *Lissachatina fulica.* Boditch in Brazil at the end of the 1990s (Teles and Fontes 2002). This terrestrial gastropod, originally from eastern Africa, is considered one of the 100 most important invasive species worldwide (Bequaert 1950, Lowe et al. 2000). The presence of *L. fulica* represents an epidemiological risk to human health, as it participates as intermediary host for several

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Fig. 1. Sampling sites, zones 1 – 4, on the Lili River in Cali, Colombia.

parasites such as *Angiostrongylus cantonensis* (Chen) and *Angiostrongylus costaricensis* Morera and Céspedes, which can cause meningoencephalitis and gastrointestinal angiostrongyliasis in humans, respectively (Maldonado et al. 2012, Londoño et al. 2013). The veterinary importance of *L. fulica* is related to the isolation of some feline nematodes such as *Aelurostrongylus abstrusus* (Railliet) and *Cruzia tentaculata* (Rudolphi) (Travassos 1917), a parasite reported in marsupials (Valente et al. 2017, Ramos-de-Souza et al. 2021).

In 2008, *L. fulica* was classified as an exotic invasive species in Colombia by the Ministry of the Environment and Sustainable Development (MAVDT 2008). The first source of infection was recorded in 2010 in the Amazon region, and the species later dispersed to 21 departments in Colombia, including the Valle del Cauca department (De La Ossa-Lacayo et al. 2012). Aquatic microenvironments in the shell of dead mollusks (gastrotelmata) and their association with mosquito species of public health importance have been little explored. Most studies on the preference of this microhabitat have been restricted to African countries and to the species in the genera *Culex*, *Aedes*, *Eretmapodites,* and *Toxorhynchites* (Van Someren et al. 1955, Rozeboom and Bridges 1972, Trips 1973, Lounibos 1980, Lounibos and Munstermann 1981, Igbinosa 1989). In the Americas, only two studies have recorded the presence of *Aedes albopictus* (Skuse) and *Limatus durhamii* (Theobald) in empty shells of the species *Pomacea insularum* (D'orbigny)*, Megalobulimus oblongus* (Müller)*, M. lorentzianus* (Doering) and *M. oblongus musculus* (Bequaert) in the state of Florida, USA, and in Argentina, respectively (Burkett-Cadena and Unnasch 2013, Mangudo et al. 2017). A better understanding of the breeding habitats used by synanthropic mosquitoes could provide epidemiological information with direct implications for public and environmental health. Therefore, the present exploratory study had the objective of determining the potential risk of empty giant African snail shells as breeding sites for mosquitoes of public health importance under field conditions in the city of Cali, Colombia.

MATERIALS AND METHODS

Study area

This study was undertaken in the city of Cali (3° 27′00″N 76°32′00″O), special district and capital of Valle del Cauca department, divided geographically into 22 communes. Cali is located at a mean height of 1,000 m and has an average temperature of 24.5 ^{0}C. The annual precipitation is 1,480 mm and the climate is warm dry tropical. Three experiments were undertaken under field conditions on the banks of the Lili River, which crosses part of commune 22 at the southern end of the city of Cali (Fig. 1). The ecological corridor in the urban part of the Lili River presents protective vegetation on its banks, forming patches of large trees, shrubs, and herbaceous and bamboo plants, which help to support a biocenosis that includes some mammal, bird, and reptile species. The Lili River basin has been highly intervened by mining activities, and we observed deposits of debris, unusable materials, and garbage in the urban part of the river course. The Lili River has a bimodal rainfall regime, with a first rainy period from April to June and a second from October to December. The dry seasons occur from January to March and from July to September. The study area comprised the right and left riverbanks of the Lili River, and each bank included

Fig. 2. Selected microenvironments for installation of empty shells of the giant African snail in zones 1-4 along the Lili River, Cali, Colombia.

two zones (Fig. 2). Selection criteria of the microenvironments for the placement of giant African snail shells were based on the following field observations on the Lili riverbanks: trees bases with presence of empty giant African snail shells and presence of resting mosquitoes. This descriptive study evaluated the variables shell size, water volume, and mosquito larvae in 3 experiments in 4 zones of the Cali riverbank according to the availability of snail shells. The methodology was based on average shell size and observations during the collection of empty snail shells in studies reported in the city of Cali.

Experiment 1: The assessment was carried out in October 2023. In this experiment, the variations of the final volume from a fixed standard volume added to all the shells evaluated and the number of mosquito larvae recovered are carried out using the following methodology. An average fixed volume of 16 ml was evaluated according to the average volume of water in the snail shells observed in the field, with a minimum size of 93 mm and a maximum size of 109 mm. A total of 16 giant African snail shells were distributed at the base of 4 trees. Five and eleven African snail shells ranged in size from 93 to 100 mm and 101 to 109 mm, respectively. The 4 giant African snail shells placed at the base of each tree were within the following size ranges: 93–113 mm, 100–106 mm, 98–107 mm, 93–117mm, with a fixed water volume of 16 ml per shell. A motorcycle tire containing 450 ml water and an 8-oz plastic cup containing 60 ml dechlorinated water were placed at the base of each selected tree as positive controls for the presence of mosquitoes. Giant African snail shells were installed 80 cm apart on the shaded base of each selected tree, 3 m away from the controls. All giant African snail shells were placed with the materials and organic matter that had accumulated inside them during the collection process. An inspection for larval and pupal presence in each microenvironment was performed every three days. After 10 days, the final volume was measured, and the entomological material from each snail shell and the accumulated organic matter were deposited in tagged 50-ml Falcon® tubes. The contents of each tube were then transferred to 8-oz (approx. 227 ml) glass containers in the laboratory and the contents were filled to a final 100 ml volume. Entomological materials were kept under laboratory conditions and observed for two weeks. Emerged adults were identified using taxonomic keys for mosquitoes by Darsie (1985). This procedure was also followed for the entomological contents recovered from the controls of the tires and plastic cups placed in each microenvironment

Experiment 2: The assessment was undertaken in November 2023. In order to identify in small African snail shells that maintain a potential epidemiological risk, with preestablished volumes of water and the number of mosquito larvae that will develop, we proceeded with the following methodology. Based on the information obtained from the previous field work we placed 5 snail shells simultaneously at each of the four selected trees, for a total of 20 snail shells. Snail shells placed at the base of the trees had the following measurements: tree no. 1, 43–82 mm; tree no. 2, 40–88) mm; tree no. 3, (42–81 mm; and tree no. 4, 48–84 mm. Sizes between 40 and 60 mm and 61 and 88 mm were distributed in 10 snail shells, respectively.

The water volumes for the different sizes of snail shells were 40–50 mm; (2.5 ml of water), 50–60 mm; (4 ml), 60–70 mm; (5 ml), 70–80 mm; (6.5 ml) and 80–90 mm; (7.5 ml). For data analyses, two categories of shell size were established: 40–60 mm and 61–80 mm. Like in the previous experiment, a positive control consisted of a motorcycle tire and a plastic cup placed as urban microenvironments of mosquito breeding at the base of the trees to establish the presence of mosquitoes in the area. After 10 days, all

Table 1. Relative abundance of mosquito species in African snail shell and containers during October 2023.

	No. shells positive per shells exposed	Mean of *Li. durhamii* per shell	Initial volume (ml)	Final volume per positive shell (ml)	*Limatus durhamii* (%)	*Aedes albopictus* (%)	*Ae. aegypti* (%)	Total (%)
Size (mm)								
93–100	4/5	10.2	16	6.5–22.0	41	4	0	45 (30)
101–109	10/10	7.7	16	13.0–45.0	77	27	1	105 (70.0)
Subtotal	14/15	—	—	—	118 (78.6)	31 (20.7)	1 (0.6)	150
Tire	—	—	450 ml	—	134	30	7	171 (70.4)
Plastic cup	—	—	60 ml	—	40	31	0	71 (29.2)
Subtotal					174 (72.0)	61 (25.2)	7 (2.9)	242
Total	15		—	—	292 (74.5)	92 (23.4)	8 (2.0)	392

entomological material, final volume of each shell and adult identification were performed under the same conditions as in experiment 1.

Experiment 3: This phase of the study was undertaken during the first week of January and May 2024, which had high rainfall. In order to know the volumes of water that accumulate in the shells of the African snails under natural conditions, the variations of the volume at the end of the evaluated period and the number of mosquito larvae, the following procedure was carried out. Seven giant African snail shells were placed at the base of each of the selected trees. Shell measurements by tree were as follows: tree no. 1 (99–112 mm); tree no. 2 (94–130 mm); tree no. 3 (93–111 mm); and tree no. 4 (84–113 mm). During the two-month study period, a total of 56 snail shells were used, of which 19 shells were between 84 and 100 mm in size and the remaining 35 between 101 and 115 mm. The water volume in each snail shell corresponded to natural accumulation during the rainy period. This phase of the study did not include controls for the presence of mosquitoes. Four size categories were established for analysis: 1) 80–90 mm, 2) 91–100 mm, 3) 101–110 mm, and 4) 111–115 mm. After 10 days, the water volume collected in each shell was measured using 50-ml centrifuge Falcon tubes. The collection of immature mosquitoes, shell volume measurements, and entomological identification were carried out as in experiments 1 and 2.

Statistical analysis: Analyses of results were performed using descriptive tables and graphs for the identification of relationships among the variables of interest. The relationship between the categorical variables, final water volume and mosquito presence was assessed using Fisher's exact test. The linear relationship between the number of mosquitoes per shell and the size and volume variables was analyzed, using Spearman's correlation test. For all tests, a P value < 0.05 denoted significant differences. Graphs and tests were created using R version 4.3.2 (R Core Team 2023).

RESULTS

Experiment 1: As shown in Table 1, a total of 150 mosquitos were identified in 15 giant African snail shells. Shell occupation was 93.3% (14/15). Under laboratory conditions, 80% of the adults emerged between days 7 and 10, which was also observed in experiments 2 and 3. The dominant mosquito species was *Li. durhamii*, with 118 specimens (71 ♀-47♂), representing 78.7% of all identified individuals. There were 31 *Ae. albopictus* specimens (20.7%, 15♀-16♂), and 1♀ *Ae. aegypti* (L.). There was an average number of 8.4 (SD = 6.2) mosquitos per shell positive for *Li. durhamii* and 3.9 (SD = 2.6) individuals in shells positive for *Ae. albopictus*. In this experiment, positive shells with sizes ranging from 93 to 100 mm were examined. The water volumes in these shells varied from 6.5 to 23.0 ml, with an average of 16.5 ml (SD = 6.8 ml). In contrast, a greater amount of water was recorded in positive shells with size 101 to 109 mm, with water volumes ranging from 13 to 45 ml and an average of 17.9 ml (SD = 10.3). This shell size also resulted in the greatest number of mosquitos (105 individuals, 70.0%) and an average of 7.7 (SD = 6.1) *Li. durhamii* specimens per shell.

The greatest number of mosquitoes per shell was 22 specimens: 14 *Li. durhamii* and 8 *Ae. albopictus*. The cooccurrence of the species *Li. durhamii* and *Ae. albopictus* was recorded in 8 shells, and 1 shell contained specimens of *Li. durhamii*, *Ae. albopictus*, and *Ae. aegypti*. The relative abundance of adult mosquitos in motorcycle tires was 134 (70♀-64♂) adult *Li. durhamii*, 30 (12♀-18♂) *Ae. albopictus*, and 7 (3 ♀-4♂) *Ae. aegypti* adults. A total of 40 (15♀-25♂) *Li. durhamii* and 31 (13♀-18♂) *Ae. albopictus* were found in plastic cups. A total of 242 adult mosquitoes were obtained in control containers, in the following proportions: *Li. durhamii* (72.0%), *Ae. albopictus* (25.2%), and *Ae. aegypti* (2.9%). In the 2 experiments, we observed an accumulation of dry leaves and unidentified dead ants in the motorcycle tires and plastic cups.

Figure 3 shows the percent distribution of mosquito species found in giant African snail shells measuring 101 to 109 mm, compared with mosquitoes obtained in the control receptacles (motorcycle tires and plastic cups). This graph shows a similar oviposition preference behavior, with 70% *Li. durhamii* and a lower proportion of *Ae. albopictus* in the three

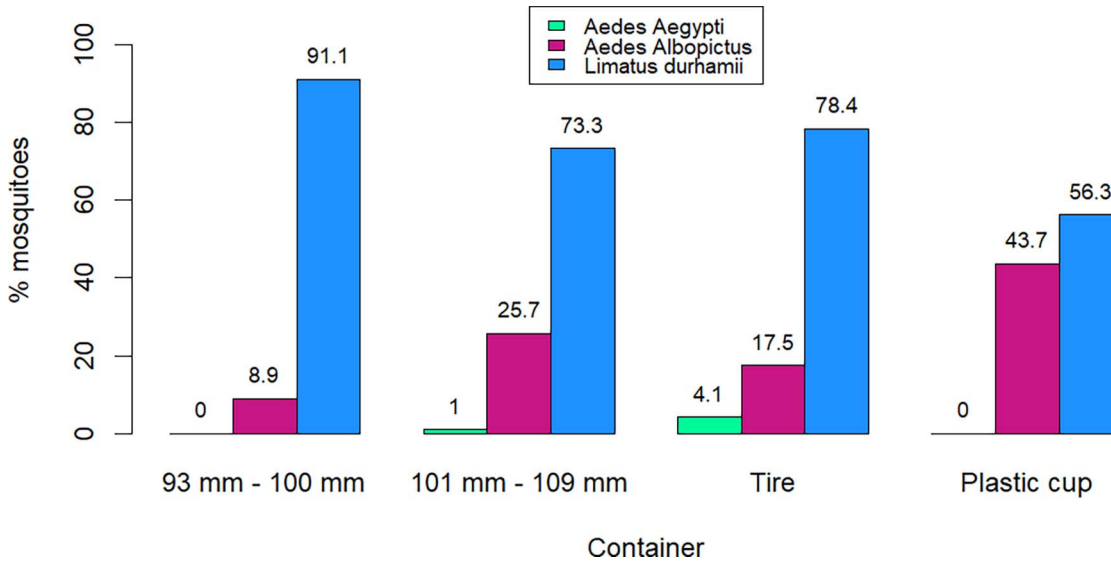

Fig. 3. Percent distribution of the three mosquito species by container type used in the study.

container types. This suggests that giant African snail shells measuring over 100 mm are as attractive as the control container (motorcycle tire) as a breeding site for these two mosquito species. A total of 392 mosquitoes were identified in the three container types evaluated.

Experiment 2: Results of this experiment are shown in Table 2. A total of 18 adult mosquitoes were identified in the 20 giant African snail shells. The dominant species was *Li. durhamii*, with 17 specimens (14♀-3 ♂); 1 ♂ Ae. *albopictus* was also obtained. There was a 35% snail shell occupation rate (7/20). Only two of the 10 (20%) giant African snail shells in the 43 to 60 mm size range showed a final volume above the initial volume. A greater percentage was observed in the 60 to 88 mm size range, with water in eight of the 10 shells (80%). A maximum of 22 ml was obtained in an 84 mm shell in this size range. Although 50% of shells still contained water after 10 days of observation, the number of mosquitoes varied between 1 and 6, with an average of 2.4 (SD = 1.8) per shell positive for *Li. durhamii*. The relative abundance of mosquitoes was low, although the experiment was begun during the rainy season in the city of Cali. A high proportion (50%) of shells without water was recorded at the end of the 10-day period, which could be associated with the shell size and location at the base of the trees. We observed the cooccurrence of *Li. durhamii* and *Ae. albopictus* in a single shell measuring over 80 mm.

A total of 177 adult mosquitoes were identified in control containers. Eighty-two (36♀-46♂) *Li. durhamii*, 59 (39♀-20♂) *Ae. albopictus*, and 8 (5♀-3♂) *Ae. aegypti* were identified in motorcycle tires; 13 (6♀-7♂) *Li. durhamii,* 14 (10♀-4♂) *Ae. albopictus*, and 1♀ *Ae. aegypti* were identified in plastic cups. No *Ae. aegypti* specimens were found in the 20 giant African snail shells placed in the study site, whereas 9 *Ae. aegypti* specimens were found in the control containers. In this experiment, 195 mosquitoes were identified in the three containers used. Fisher's test showed that the relationship between the final water volume in snail shells and the presence of mosquitoes in the 61-88 mm shell size was 0.033. Therefore, the final volume variable was significant to explain the rate of mosquitos per shell.

Table 2. Relative abundance of mosquito species in African snail shell and containers during November 2023.

	No. shells positive per shells exposed	Initial volume per shell (ml)	No. shells with final volume of water	*Limatus durhamii* (%)	*Ades. albopictus* (%)	*Ae. aegypti* (%)	Total (%)
Size (mm)							
40–61	1/10	2.5 - 4.0 - 4.5	2 /10	1	0	0	1
61–88	6/10	5.5 - 6.5 - 7.5	8/10	16	1	0	17
Subtotal	7/20			17 (94.4)	1	0	18 (9.2)
Tire		450		82	59	8	149
Plastic cup		60		13	14	1♀	28
Subtotal				95 (53.6)	73 (41.2)	9 (5.0)	177
Total				112 (57.4)	74 (38.0)	9 (4.6)	195 (90.8)

Table 3. Relative abundance of mosquito species in African snail shell during January and May 2024.

	No. shells positive per shells exposed	No. shells with water	Mean of mosquitoes per positive shell	Volume (ml) per positive shell	*Limatus durhamii* (%)	*Aedes albopictus* (%)	*Ae. aegypti* (%)	Total (%)
Size (mm)								
80–90	1/4	2.0	1	0–4.0	1	0	0	1 (0.005)
91–100	6/15	8.0	3.3	1–11.0	19	1	0	20 (11.7)
101–110	15/27	22.0	5.7	4–25.0	41	39	5	85 (50.0)
111–115	7/8	7.0	9.1	1–27.5	37	27	0	64 (37.6)
Total	29/54 (53.7)	39 (72%)			98 (57.9)	67 (42.1)	5 (2.9)	170

Experiment 3: Results of this experiment are shown in Table 3. Water was recorded in 39 of 54 giant African snail shells collected (72.2%). A total of 29 shells contained immature mosquito stages, with a total of 170 mosquitoes identified. The dominant species was *Li. durhamii* (57.9%) with 98 individuals (64♀-34♂). There were also 67 (21♀-46♂) *Ae. albopictus* individuals (39.4%) and 5 *Ae. aegypti* (3♀-2♂) individuals (2.9%). Giant African snail shells containing water had 56.4% *Li. durhamii* and 25.6% *Ae. albopictus* specimens. Of the 39 snail shells containing water, only 29 shells (74.3%) were positive for mosquito larvae. The mean size of shells was 100.7 mm. The average number of mosquitoes per shell increased as the size range of the shells increased. The mean number of mosquito larvae per shell in the 101–110 mm and 111–115 mm size ranges was 5.7 (SD = 5.4) and 9.1 (SD = 8.8), respectively. The average number of *Li. durhamii* per shell positive for mosquito larvae was 4.3 (SD = 3.8), with the number of individuals ranging between 1 and 15. The average volume of water in snail shells positive for mosquito larvae was 9.4 ml (SD = 6.7 ml). Water volume varied from 1 ml with presence of one *Li. durhamii* larva to a maximum of 27.5 ml with 26 *Ae. albopictus* larvae. We observed the co-occurrence of *Li. durhamii* and *Ae. albopictus* in three giant African snail shells measuring over 100 mm, and there was cooccurrence of *Li. durhamii*, *Ae. albopictus,* and *Ae. aegypti* in one snail shell. Spearman's correlation test was used to analyze the linear relationship between the number of mosquitoes per snail shell; shell size and volume; P values obtained were significant ($P = 0.040$ for size, Figure 4, and $P = 0.014$ for volume, Fig. 5). Additionally, we found, as expected, that the volume and shell size have a positive association (Fig. 6). Under the conditions of this experiment, these results suggested that water storage capacity was greater at larger shell sizes, with abiotic conditions that allowed greater larval density.

Finally, a total of 757 mosquitoes were identified in this study in the three evaluated containers during the three experiments, as follows: 233 *Li. durhamii*, 99 *Ae. albopictus* and 6 *Ae. aegypti* in giant African snail shells, 216 *Li. durhamii*, 89 *Ae. albopictus*, and *15 Ae. aegypti* in motorcycle tires; and 53 *Li. durhamii*, 45 *Ae. albopictus,* and one *Ae. aegypti* specimen in plastic cups. The percent distribution in the three containers was as follows: *Li. durhamii* 66.3% (n = 502), *Ae. albopictus* 30.8% (n = 233), and *Ae. aegypti* 2.9% (n = 22).

DISCUSSION

Information on the habitat of the larvae of medically important mosquito species is essential to understand

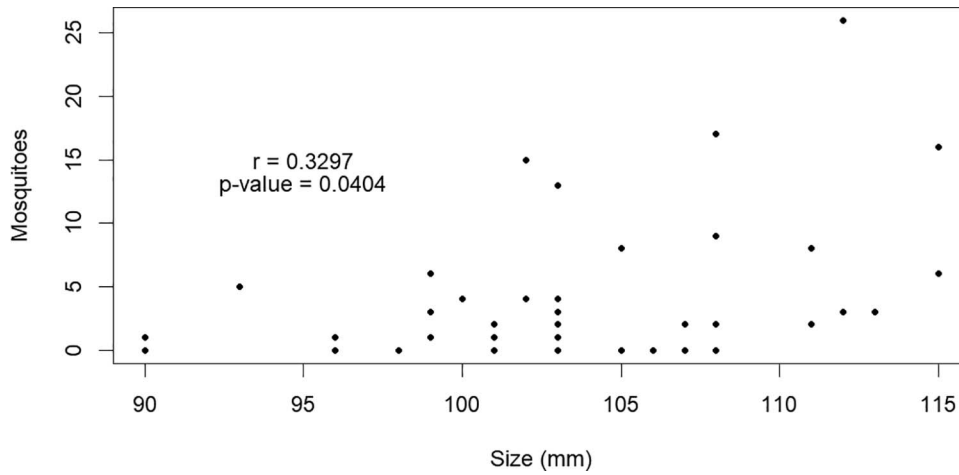

Fig. 4. Relationship between mosquito numbers and snail shell size (r = 0.3297; $P = 0.0404$).

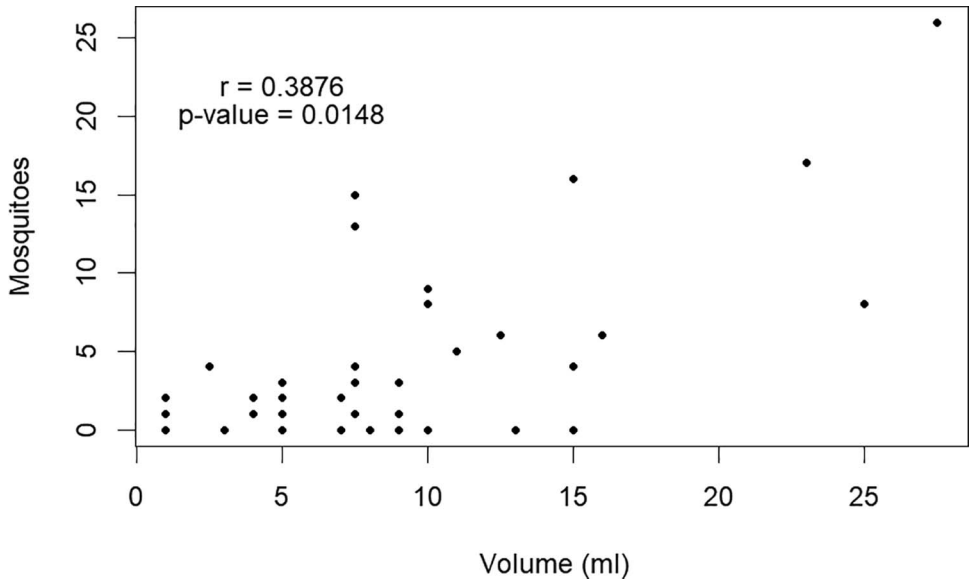

Fig. 5. Relationship between mosquito numbers and snail shell volume (r = 0.3876; $P = 0.0148$).

population dynamics and to design cost-effective vector control strategies. During the three experiments using empty giant African snail shells, motorcycle tires, and plastic cups undertaken on the banks of the Lili River, we observed the presence of three species that are important vectors in public health. *Li. durhamii* was the dominant species in this study. This species is distributed in South America from Argentina to Central America, including Mexico, and it is also found in West Indies countries such as Trinidad and Tobago. Taxonomically, it is included in the Sabhetine tribe, Subfamily Culicinae. Due to its ecological plasticity, this species has adapted to anthropogenic environments and its immature stages have been collected in bamboo cavities, tree holes, coconut husks, cocoa pods, snail shells, rock hollows, tires, and a wide variety of small artificial containers (Campos et al. 2011, Chaverri et al. 2018). Although there are several studies on the bionomy of *Li. durhamii*, research on the ecology of breeding sites in snail shells are limited to three studies undertaken in 2012 in Yungas, Argentina, an area of subtropical rainforest (Mangudo et al. 2017). The snail species *M. oblongus*, *M. lorentzianus*, and *M. oblongus musculus* were recorded in the three studied sites. We compare the results of that study with results of experiment 3 for *Li. durhamii,* given the field conditions in which the tests were performed. Some differences in results could be related to the shell sizes in the two studies. The average number of *Li. durhamii* larvae was 11.6 larvae for species in the genus *Megalobulimus*,

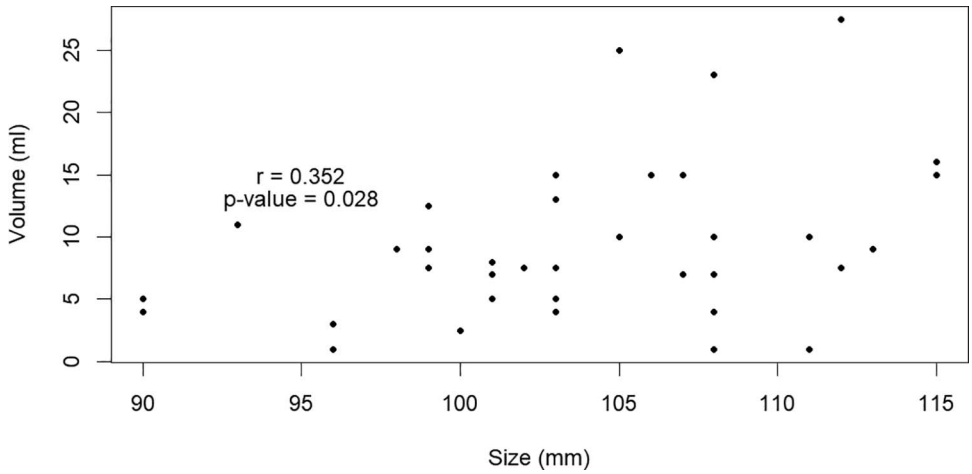

Fig. 6. Relationship between snail shell volume and shell size (r = 0.3352; $P = 0.028$).

compared with 4.3 *Li. durhamii* mosquitos per giant African snail shell in experiment 3. The study in Argentina reported between 1 and 27 immature larvae per snail shell, in contrast with results in experiment 3, in which between 1 and 16 *Li. durhamii* mosquitoes were found. This difference could be because of the simultaneous presence of sympatric species such as *Ae. albopictus* and *Ae. aegypti*, which could be competing for breeding site selection, space, and nutrient content in the shells. The average water volume recorded of 9.4 ml per giant African snail shell (range = 1 to 27.5 ml per shell) was greater in experiment 3 than what was found for the species in the *Megalobulimus* genus (mean = 3.5 ml and range = 0.5 to 10 ml per shell).

The epidemiological importance of the *Li. durhamii* findings in this study resides in vectorial susceptibility, with the isolation of viruses that comprise serogroups Guama and Group C of the genus *Orthobunyavirus* (subfamily *Bunyaviridae*) in the Amazon region of Peru and during entomovirus monitoring in an urban forestry reserve in Brazil, with the Zika virus (Turell et al. 2005, Barrio-Nuevo et al. 2020). *Li. durhamii* has a wide distribution in Colombia and recent studies identified this mosquito species in 15 ecoregions, with presence of mosquitoes from the Sabhetini tribe (Naranjo-Díaz et al. 2022); however, research on the role of *Li. durhamii* in the transmission of some flaviviruses with potential health risk have not been reported. Another vector of interest was *Ae. albopictus*, an invasive species from southeast Asia, whose geographical distribution includes all continents except Antarctica. Its introduction to the Americas was associated with the international trade in vehicle tires, and there is information on its presence in 21 countries (Huang 1968, Garcia-Rejon et al. 2021). In Colombia, it was reported for the first time in the city of Leticia in 1998 and in the city of Cali in 2006. It is currently recorded in 23 of 32 departments in Colombia (Vélez et al. 1998, Cuéllar-Jiménez et al. 2007, Hoyos et al. 2023). In the Americas and Colombia, it is considered a competent vector in the transmission of the arboviruses dengue, Zika, and chikungunya (Martínez et al. 2020, Garcia-Rejon et al. 2021). *Aedes albopictus* shares the same ecotypes as *Li. durhamii*. Its success as an invasive species and ability to displace other species is linked with a gonotrophic cycle that is shorter than that of *Ae. aegypti* by approximately 3.2 to 3.7 days (Casas-Martínez et al. 2020) and the ability to lay eggs without the need for blood intake (Klowden and Chambers 1992). *Aedes albopictus* larvae have the ability to decelerate growth and resist hunger under limited food conditions (Barrera 1996). Additionally, eggs can enter diapause because of environmental conditions such as temperature, prolonged dry periods, and increased solar radiation, which is a characteristic that *Ae. aegypti* does not share (Lacour et al. 2014).

The productivity of *Ae. albopictus* larvae was reported for *P. insularum* aquatic snails on the Hillsborough River banks in the state of Florida, USA, in 2012. A total of 108 shells were collected over 3.5 km, only 25 of which were positive for mosquitoes, yielding 367 *Ae. albopictus* larvae. An average of 14.6 *Ae. albopictus* larvae per shell and a maximum of 56 larvae in a shell containing 15 ml of water were recorded. These results on the Hillsborough River were greater than those found in experiment 3 in this study, with a total of 67 *Ae. albopictus* adults in nine shells positive for mosquito presence, and an average of 6.7 mosquitoes. The maximum number of mosquitoes in one snail shell was 26 specimens in 27.5 ml of water. Another investigation where the objective was entomological monitoring in natural and artificial containers, Rozeboom and Bridges (1972). found the dominant presence of *Ae. albopictus* in *L. fulica* shells on Guam Island, in the Mariana Islands archipelago in Oceania. In that study, the presence of only 50 *Ae. albopictus* larvae in 9 African snail shells was reported.

Aedes aegypti is a domestic species originally from sub-Saharan Africa that was introduced to the Americas during the slave trade in the 16th and 17th centuries. It is distributed over 80% of the Colombian territory, in populations located up to 2,300 m (Ruiz-López et al. 2016, Powell et al. 2018). *Ae*des *aegypti* is considered a primary vector in the transmission of dengue, chikungunya and Zika viruses, and a principal urban vector of yellow fever (Souza-Neto et al. 2019). The evolutionary history of *Ae. aegypti* is strongly linked to human settlements and it probably evolved and adapted to the domestic environment (Powell et al. 2018). The domestic habitat and most artificial containers built by humans using plastic, metal, mud, glass, cement, clay, tires, etc., are considered the main breeding sites for *Ae. aegypti*, although natural containers should also be taken into account (Chadee et al. 1998, Maciel-de-Freitas et al. 2007). The presence of *Ae. aegypti* larvae in *L. fulica* shells was also described in a study undertaken in the city of Dar es Salaam and in the Msasani Peninsula of Tanzania in 1970. In that study, three species were identified in 207 shells: *Ae. aegypti*, *Ae. simpsoni* Theobald, and *Eretmapodites quinquevittatus* Theobald. The average size of snail shells was 95.9 mm, and water volumes per shell ranged between 140 and 250 ml in the two study sites, with an average of 56.5 ml. The number of larvae per shell ranged from 1 to 35, with records of 40 *Er. quinquevittatus,* 14 *Ae. simpsoni*, and 32 *Ae. aegypti* specimens per shell. The latter species was dominant, with 223 specimens. In experiment 3 in this study, the average size per shell was 100.7 mm. The number of larvae per shell ranged from 1 to 26 individuals and the average volume per shell positive for mosquito presence was 9.4 ml. The relative density of *Ae. aegypti* in experiment 3 was low, and only one 108 mm shell with a volume of 23 ml was recorded, with only five individuals.

The low density of *Ae. aegypti* in this research, in snail shell containers, motorcycle tires and plastic cups, may be related to the requirements of this species during the selection of oviposition sites such as: their occupancy, biological conditions of the water and availability of food. (Chadee 2009). Additionally,

the low presence of *Ae. aegypti* could be explained if we take into account the domesticated behavior of oviposition inside and outside housing and the offer of rainwater drains in the streets surrounding the study area, considered one of the greatest artificial hatcheries in the city that offers a high volume of water and available organic matter year-round (Obando et al. 2007). The entomological percentages of *Li. durhamii* (66%) and *Ae. albopictus* (32.7%) recorded in the motorcycle tire and plastic cup containers in the first 2 experiments confirmed the ecological plasticity of the 2 species.

Results of a study on the morphological variations of giant African snails undertaken in Cali in 2018 in different communes found that the average size of giant African snail shells was 53,71 ± 12,97 mm, classified as a young - adult population (Patiño-Montoya et al. 2018). These observations on the average size of snail shells were related to, among other factors, the continuous collection of live giant African snails by the environmental authorities of the city of Cali. These results on the average size of snail shells, according to the authors, are due, among other factors, to the continuous collection of live snails by the environmental authorities of Cali. Comparison of the above information and the potential of snail shells larger than 80 mm suggests that monitoring of African snail empty shells should be included in vector control activities.

Finally, the results of our exploratory research raise the need to address control methodologies for giant African snail shells that could include 1) continuing the systematic collection by environmental authorities in municipalities endemic to this invasive mollusk, and 2) creation of community cooperatives, with the support of local mayors, for the collection and marketing of snail shells, which are currently used to extract calcium carbonate and its derivatives for medical and industrial applications.

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REFERENCES CITED

Alarcón-Elbal PM, Rodríguez-Sosa MA, Ruiz-Matuk C, Tapia L, Arredondo Abreu CA, Fernández González AA, Rodríguez Lauzurique RM, Paulino-Ramírez R. 2021. Breeding Sites of Synanthropic Mosquitoes in Zika-Affected Areas of the Dominican Republic. *J Am Mosq Control Assoc*. 37:10–19. https://doi.org/10.2987/20-6953.1

Barrera R. 1996. Competition and resistance to starvation in larvae of container-inhabiting mosquitoes. *Ecol Entomol* 21:117–127. https://doi.org/10.1111/j.1365-2311.1996.tb01178.x

Barrio-Nuevo KM, Cunha MS, Luchs A, Fernandes A, Rocco IM, Mucci LF, de Souza RP, Medeiros-Sousa AR, Ceretti-Junior W, Marrelli MT. 2020. Detection of Zika and dengue viruses in wild-caught mosquitoes collected during field surveillance in an environmental protection area in São Paulo, Brazil. *PLoS One* 16:15. https://doi.org/10.1371/journal.pone.0227239

Bequaert JC. 1950. Studies in the Achatininae, a group of African land snails. *Bull Mus Comp Zool* 105:1–216.

Burkett-Cadena ND. and Unnasch TR. 2013. Apple snails and tiger mosquitoes: a curious association between two invasive species in Florida, USA. Paper presented at Entomology 2013, 61st Annual Meeting of the Entomological Society of America, 10–13 November 2013, Austin Convention Center, Austin, Texas. Available at: https://esa.confex.com/esa/2013/webprogram/Session20756.html

Campos RE, Spinelli G, Mogi M. 2011. Culicidae and Ceratopogonidae (Diptera: Nematocera) inhabiting phytotelmata in Iguazú National Park, Misiones Province, subtropical Argentina. *Revista de la Sociedad Entomológica Argentina* 70:111–118.

Casas-Martínez M, Tamayo-Domínguez R, Bond-Compeán JG, Rojas JC, Weber M, Ulloa-García. 2020. *Aedes*. oogenic development and gonotrophic cycle of *Aedes aegypti* and *Aedes albopictus* in laboratory. *Salud Publica Mex* 62:372–378. https://doi.org/10.21149/10164

Chadee DD. 2009. Oviposition strategies adopted by gravid *Aedes aegypti* (L.) (Diptera: Culicidae) as detected by ovitraps in Trinidad, West Indies (2002-2006). *Acta Tropica* 111:279–283. https://doi.org/10.1016/j.actatropica.2009.05.012

Chadee DD, Ward RA, Novak RJ. 1998. Natural habitats of *Aedes Aegypti* in the Caribbean–a review. *J Am Mosq Control Assoc* 14:5–11.

Chaverri LG, Dillenbeck C, Lewis D, Rivera C, Romero LM, Chaves LF. 2018. Mosquito species (Diptera: Culicidae) diversity from ovitraps in a Mesoamerican tropical rainforest. *J Med Entomol* 55:646–653. https://doi.org/10.1093/jme/tjx254

Chaves LF, Hamer GL, Walker ED, Brown WM, Ruiz MO, Kitron UD. 2011. Climatic variability and landscape heterogeneity impact urban mosquito diversity and vector abundance and infection. *Ecosphere* 2:art70. https://doi.org/10.1890/es11-00088.1

Cuéllar-Jiménez, ME, Velásquez-Escobar OL, González-Obando R, Morales-Reichmann CA. 2007. Detección de *Aedes albopictus* (Skuse) (Diptera: Culicidae) en la ciudad de Cali, Valle del Cauca, Colombia. *Biomédica*. 27:273–279. https://doi.org/10.7705/biomedica.v27i2.224

Darsie R. 1985. The mosquitoes of Argentina: Part I. Keys for identification of adult females and fourth stage larvae in English and Spanish (Diptera: Culicidae). *Mosq Syst* 17:153–253.

De La Ossa-Lacayo A, De La Ossa VJ, Lasso CA. 2012. Registro del caracol africano gigante *Achatina fulica* (Bowdich 1822) (Mollusca: Gastropoda-Achatinidae) en Sincelejo, costa Caribe de Colombia. *Biota Colombiana* 13:247–252. https://doi.org/10.21068/bc.v13i2.270

Fontenille D, and Toto JC. 2001. *Aedes (Stegomyia) albopictus* (Skuse), a potential new dengue vector in Southern Cameroon. *Emerg Infec Dis* 7:1066–1067. https://doi.org/10.3201/eid0706.010631

Garcia-Rejon J, Navarro JC, Cigarroa-Toledo N, Baak-Baak CM. 2021. An updated review of the invasive aedes

albopictus in the Americas; geographical distribution, host feeding patterns, arbovirus infection, and the potential for vertical transmission of dengue. *Virus Insects* 12:967. https://doi.org/10.3390/insects12110967

Gubler DJ. 2011. Dengue, urbanization and globalization: the unholy trinity of the 21st century. *Trop Med Health* 39:3–11. https://doi.org/10.2149/tmh.2011-s05

Hoyos P A, Rúa-Uribe G, Wolff M. 2023. *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae): nuevos registros para Colombia. *Revista Chilena de Entomología*. 49:825–842. https://doi.org/10.35249/rche.49.4.23.17

Huang YM. 1968. Neotype designation for *Aedes (Stegomyia) albopictus* (Skuse) (Diptera: Culicidae). *Proc Entomol Soc Wash* 70:297–302.

Igbinosa IB. 1989. Investigations on the breeding site preferences of mosquitoes in Ekpoma. Nigeria *J Appl. Entomol*. 107:325–330. https://doi.org/10.1111/j.1439-0418.1989.tb00264.x

Johnson MTJ, Munshi-South J. 2017. Evolution of life in urban environments. *Science* 358:eaam8327. https://doi.org/10.1126/science.aam8327

Klowden MJ, Chambers GM. 1992. Reproductive and metabolic differences between *Aedes aegypti* and *Ae. albopictus* (Diptera: Culicidae). *J Med Entomol* 29:467–471. https://doi.org/10.1093/jmedent/29.3.467

Lacour G, Vernicho F, Cadilhac N, Boyer S, Lagneau C, Hance T. 2014. When mothers anticipate effects of the prediapause stage on embryo development time and of maternal photoperiod on eggs of a temperate and a tropical strains of *Aedes albopictus* (Diptera: Culicidae). *J Insect Phy* 71:87–96. https://doi.org/10.1016/j.jinsphys.2014.10.008

Londoño JD, Zamora A, Osorio J. 2013. *Angiostrongylus Cantonensis* y el caracol africano gigante como causantes de meningitis eosinofilica. *Revista Facultad de Salud* 5:61–69. https://doi.org/10.25054/rfs.v5i2.143

Lounibos LP. 1980. The bionomics of three sympatric *Eretmapodites* (Diptera: Culicidae) at the Kenya coast. *Bull Entomol Res* 70:309–320. https://doi.org/10.1017/s0007485300007598

Lounibos LP, Munstermann LE. 1981. Ecological and genetic separation of three sympatric species of *Aedes* (Diptera: Culicidae) from the Kenya coast. *Bull Entomol Res* 71:639–648. https://doi.org/10.1017/s0007485300010166

Lowe S, Browne M, Boudjelas S, De Poorter M. 2000. *100 of the world's worst invasive alien species: a selection from the Global Invasive Species Database.* Published by The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), 12pp.

Maciel-de-Freitas R, Marques WA, Peres RC, Cunha SP, de Oliveira RL. 2007. Variation in *Aedes aegypti* (Diptera: Culicidae) container productivity in a slum and a suburban district of Rio de Janeiro during dry and wet seasons. *Mem Inst Oswaldo Cruz* 102:489–496. https://doi.org/10.1590/s0074-02762007005000056

Maldonado A, Simoes R, Thiengo S. 2012. Angiostrongyliasis in the Americas. In: Lorenzo-Morales J, editor. Zoonosis. Brasil: En InTech eBooks. https://doi.org/10.5772/38632

Mangudo C, Campos RE, Rossi GC, Gleiser RM. 2017. Snail shells as larval habitat of *Limatus durhamii* (Diptera: Culicidae) in the Yungas of Argentina. *Acta Tropica* 167:204–207. https://doi.org/10.1016/j.actatropica.2016.12.036

Martínez D, Hernández C, Muñoz M, Armesto Y, Cuervo A, Ramírez JD. 2020. Identification of *Aedes* (Diptera: Culicidae) species and arboviruses circulating in Arauca, Eastern Colombia. *Front Ecol Evol* 8:602190. https://doi.org/10.3389/fevo.2020.602190

MAVDT [Ministerio de Ambiente, Vivienda y Desarrollo Territorial]. 2008. Resolución Número 0848 del 23 de mayo de 2008. Por el cual se declaran unas especies exóticas como invasoras y se señalan las especies introducidas irregularmente al país que pueden ser objeto de cría en ciclo cerrado y se adoptan otras determinaciones. Ministerio de Ambiente, Vivienda y Desarrollo Territorial. MAVDT, Colombia. Available from: https://www.minambiente.gov.co/documento-entidad/resolucion-0848-de-2008/

McKinney M. 2001. Effects of human population, area, and time on non-native plant and fish diversity in the United States. *Biological Conservation* 100:243–252. https://doi.org/10.1016/s0006-3207(01)00027-1

Naranjo-Díaz N, Suaza-Vasco J, Pineda-Angel J, Uribe S. 2022. New records of *Sabethini* (Diptera: Culicidae) from Colombia. *Biodiversity Data J* 10:e68413. https://doi.org/10.3897/bdj.10.e68413

Nihad M, Rohini PD, Sutharsan G, Anagha A. PK, Sumitha MK, Shanmuga Priya A, Rahul P, Sasikumar V, Dasgupta S, Krishnan J and Ishtiaq F. 2022. Island biogeography and human practices drive ecological connectivity in mosquito species richness in Lakshadweep Archipelago. *Scientific Reports*. 12:8060. https://doi.org/10.1038/s41598-022-11898-y

Obando RG, Gamboa F, Perafán O, Suárez MF, Lerma JM. 2007. Experiencia de un análisis entomológico de criaderos de *Aedes aegypti* y *Culex quinquefasciatus* en Cali, Colombia. *Revista Colombiana de Entomologia* 33:148–156.

Patiño-Montoya A, Murillo O, Giraldo A. 2018. Morphological variation in the populations of an invasive species: the Giant African Snail *Achatina fulica* (Bowdich, 1822) (Mollusca: Gastropoda-Achatinidae) in the department of Valle del Cauca, Colombia. *Biota Colombiana* 19(1):112–122. https://doi.org/10.21068/2018v19n01a07

Powell J, Gloria-Soria A, Kotsakiozi P. 2018. Recent history of *Aedes aegypti*: vector genomics and epidemiology records. *Bioscience* 68:854–860. https://doi.org/10.1093/biosci/biy119

R Core Team 2023. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/

Ramos-de-Souza J, Maldonado A, Jr, Vilela RV, Andrade-Silva BE, Barbosa HS, Gomes SR, Thiengo SC.2021. First report of the nematode *Cruzia tentaculata* using molluscs as natural intermediate hosts, based on morphology and genetic markers. *Int J Parasitol Parasites Wildl* 15:105–111. https://doi.org/10.1016/j.ijppaw.2021.02.013

Rejmankova E, Grieco J, Achee N, Roberts DR. 2013. Ecology of larval habitats. In: Manguin S, editor. *Anopheles* mosquitoes: new insights into malaria vectors 9th. *InTech*Open. p. 397–446. https://doi.org/10.5772/55229

Rozeboom LE, Bridges JR. 1972. Relative population densities of *Aedes albopictus* and *Aedes guamensis* on Guam. *Bull WHO* 46:477–483.

Ruiz-López F, González-Mazo A, Vélez-Mira A, Gomez FG, Zuleta L, Uribe S, Vélez ID. 2016. Presencia de *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762) y su infección natural con el virus del dengue en alturas no registradas para Colombia. *Biomedica* 36:303–308. https://doi.org/10.7705/biomedica.v36i2.3301

Shochat E, Warren PS, Faeth SH, McIntyre NE, Hope D. 2006. From patterns to emerging processes in mechanistic

urban ecology. *Trends Ecol Evol* 21:186–191. https://doi.org/10.1016/j.tree.2005.11.019

Souza-Neto JA, Powell JR, Bonizzoni M. 2019. *Aedes aegypti* vector competence studies: a review. *Infect Genet Evol* 67:191–209. https://doi.org/10.1016/j.meegid.2018.11.009.

Teles HMS, Fontes LR. 2002. Implicações da introdução e dispersão de *Achatina fulica* Bowdich, 1822 no Brasil. *Boletim do Instituto Adolfo Lutz* 12:3–5.

Trips M. 1973.Ecological studies on the breeding of *Aedes aegypti* and other mosquito in shells of the giant African snail, *Achatina fulica*. *Bull World Hlth Org* 48:447–453.

Turell MJ, O'Guinn, ML, Jones JR, Sardelis MR, Dohm DJ, Watts DM, Fernandez R, Da Rosa AT, Guzman H, Tesh R, Rossi C., Ludwig, GV, Mangiafico JA, Kondig JP, Wasieloski LP, Pecor JE, Zyzak M, Schoeler GB, Mores CN, Calampa C, Lee JC, Klein TA. 2005. Isolation of viruses from mosquitoes (Diptera: Culicidae) collected in the Amazon Basin Region of Peru. *J Med Entomol* 42:891–898. https://doi.org/10.1603/0022-2585(2005)042

Valente R, Diaz JI, Salomón OD, Navone GT. 2017. Natural infection of the feline lungworm *Aelurostrongylus abstrusus* in the invasive snail *Achatina fulica* from Argentina. *Vet Parasitol* 23:17–19. https://doi.org/10.1016/j.vetpar.2017.01.006

Van Someren ECC, C Teesdale, M Furlong. 1955. The mosquitos of the Kenya coast; records of occurrence, behaviour and habitat. *Bull Entomol Res* 46:463–493. https://doi.org/10.1017/s0007485300039481

Vélez ID, Quiñones ML, Suarez M, Olano V, Murcia LM, Correa E, Arévalo C, Pérez L, Brochero H, Morales A. 1998. Presencia de *Aedes albopictus* en Leticia, Amazonas, Colombia. *Biomédica* 18:192–198. https://doi.org/10.7705/biomedica.v18i3.990

Weaver SC. 2013. Urbanization and geographic expansion of zoonotic arboviral diseases: mechanisms and potential strategies for prevention. *Trends Microbiol* 21:360–363. https://doi.org/10.1016/j.tim.2013.03.003

WHO [World Health Organization]. 2022. World malaria report 2022. World Health Organization. Available from: https://www.who.int/publications/i/item/9789240064898.

WHO [World Health Organization]. 2017. Global vector control response 2017–2030. Available from: https://www.who.int/publications/i/item/9789241512978

Zahouli JBZ, Benjamin K, Müller P, Malone D Yao, Utzinger J. 2017. Urbanization is a main driver for the larval ecology of *Aedes* mosquitoes in arbovirus-endemic settings in south-eastern Côte d'Ivoire. *PLoS Negl Trop Dis*:11. https://doi.org/10.1371/journal.pntd.000575