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A importância dos remanescentes florestais na prevenção de arboviroses urbanas

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Dissertation submitted to the Ecology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Sérvio Pontes Ribeiro

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A vida que chamo de minha: minha mãe, que sob muito sol me permitiu chegar até aqui, na sombra.

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“*O mais importante é inventar o Brasil que nós queremos*”.
(Darcy ribeiro)

ABSTRACT

MOREIRA, Rafaela Luiza, M.Sc., Universidade Federal de Viçosa, February, 2025. **Montane urban forests play a key role in mitigating arbovirus transmission**. Adviser: Sérvio Pontes Ribeiro.

The mosquitoes *Aedes aegypti* (Linnaeus, 1762) and *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae), the primary vectors of urban arboviruses, are invasive and synanthropic species with distinct habitat preferences. Their oviposition success is influenced by the degree of urban vegetation, shaping their distribution across different landscapes. However, the influence of urban forests on the distribution of these species, as well as the effects of altitude and associated climatic variations, remains poorly understood. To monitor their distribution across different urban biotopes, ovitraps were installed during the 2022 rainy season in three distinct environments in Ouro Preto and Mariana, Minas Gerais, Brazil: urban forest, landscaped residential area, and highly urbanized area. This sampling design replicated part of a study conducted a decade earlier in these cities, where these species likely established due to rising temperatures in the early 21st century.
Our results indicate that *Aedes* vector distribution and abundance are influenced by biotope type, city, and year ($p < 0.001$). *Ae. aegypti* was the most abundant species, particularly in urbanized areas, while *Ae. albopictus* exhibited a more homogeneous distribution across the urban landscape. Between 2012 and 2022, *Ae. aegypti* increased by 1189.7%, with the highest growth in Ouro Preto (74,300.0%). Ae. albopictus maintained overall stable abundance (+5.5%), with a 14,700.0% increase in Ouro Preto and a 31.5% decrease in Mariana. These findings suggest that well-managed urban green spaces may be essential for mitigating the risk of arbovirus transmission, including dengue, Zika, and chikungunya.

Keywords: *Aedes*; Insects vectors of tropical diseases; Global Warming; Urban Ecology; Neglected diseases

RESUMO

MOREIRA, Rafaela Luiza, M.Sc., Universidade Federal de Viçosa, fevereiro de 2025. **A importância dos remanescentes florestais na prevenção de arboviroses urbanas**. Orientador: Sérvio Pontes Ribeiro.

Os mosquitos *Aedes aegypti* (Linnaeus, 1762) e *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae), principais vetores de arboviroses urbanas, são espécies invasoras e sinantrópicas com tendências distintas de habitat. Seu sucesso de oviposição é influenciado pelo grau de vegetação urbana, moldando sua distribuição em diferentes paisagens. No entanto, a influência das florestas urbanas na distribuição dessas espécies, bem como os efeitos da altitude e variações climáticas associadas, ainda são pouco compreendidos. Para monitorar sua distribuição em diferentes biótopos urbanos, ovitrampas foram instaladas na estação chuvosa de 2022 em três ambientes distintos em Ouro Preto e Mariana, Minas Gerais, Brasil: floresta urbana, área residencial ajardinada e área altamente urbanizada. Esse desenho amostral replicou parte de um estudo conduzido 10 anos antes nessas cidades, onde essas espécies provavelmente se estabeleceram devido ao aumento das temperaturas no início do século XXI.

Nossos resultados indicam que a distribuição e abundância dos vetores *Aedes* são influenciadas pelo tipo de biótopo, cidade e ano ($p < 0,001$). *Ae. aegypti* foi a espécie mais abundante, sobretudo em áreas urbanizadas, enquanto Ae. albopictus apresentou distribuição homogênea na paisagem urbana. Entre 2012 e 2022, Ae. aegypti aumentou 1189,7%, com maior crescimento em Ouro Preto (74.300,0%). *Ae. albopictus* manteve uma abundância geral estável (+5,5%), com aumento de 14.700,0% em Ouro Preto e redução de 31,5% em Mariana. Esses achados sugerem que espaços verdes urbanos bem manejados podem ser fundamentais para mitigar o risco de transmissão de arboviroses como dengue, Zika e chikungunya.

Palavras-chave: *Aedes*; Insetos vetores de doenças tropicais; Aquecimento global; Ecologia urbana; Doenças negligenciadas

SUMÁRIO

Revisão de literatura

Mosquitos da família Culicidae (Diptera, Nematocera) são os principais vetores artrópodes envolvidos no ciclo de transmissão de arbovírus (WEAVER, 2006). Entretanto, das mais de 3700 espécies descritas apenas uma pequena minoria é apontada como de importância médica e sanitária (POWELL, 2019). Entre essas, destacam-se espécies domésticas como *Aedes aegypti* e *Culex quinquefasciatus* (complexo *pipiens*) que evoluíram em estreita associação com seres humanos (FARAJOLLAHI *et al.*, 2011; POWELL; TABACHNICK, 2013). Esse fenômeno se deve a processos coevolutivos, por longos ou curtos períodos de tempo, que moldaram as interações entre o vetor, o patógeno e o hospedeiro vertebrado (WEAVER, 2006; WOOLHOUSE *et al.*, 2002).

O ciclo de transmissão de arbovírus envolve não apenas a capacidade do patógeno de sobreviver e se replicar no organismo do mosquito, mas também o desenvolvimento de características comportamentais e fisiológicas nos vetores que facilitam a disseminação dos patógenos nas populações humanas e animais (LEFEVRE *et al.*, 2017; POWELL, 2019). De origem zoonótica, a maioria dos arbovírus tem ciclos de transmissão ancestral na vida selvagem e, através de três mecanismos distintos, tem nos humanos e animais domésticos um hospedeiro acidental ou sem saída (CAMPOS *et al.*, 2023; YOUNG, 2018).

O primeiro mecanismo é o transbordamento direto, em que um vetor enzoótico – um artrópode que vive em um ecossistema natural, ou ponte – um artrópode que pode se mover entre diferentes ecossistemas, transmite o vírus de um hospedeiro enzoótico – animal silvestre que mantém o vírus na natureza, para o ser humano. Um exemplo disso é o vírus do Nilo Ocidental (WNV), transmitido por mosquitos do gênero *Culex*, que acompanha a expansão geográfica das Américas (WEAVER; REISEN, 2010). O segundo mecanismo é a amplificação em animais domesticados, que atuam como hospedeiros intermediários, seguida do transbordamento para os humanos. Exemplos desse mecanismo é a Encefalite Japonesa (JEV), um arbovírus aviário transmitido por *Culex*, e amplificado em suínos; e o vírus da febre do Vale do Rift (RVFV), amplificado em ovinos, bovinos e outros animais domesticados, transmitido por mosquitos dos gêneros *Aedes* e *Culex* (WEAVER *et al.*, 2017). O terceiro mecanismo é a transição do ciclo enzoótico para um ciclo predominantemente humano, onde os humanos servem como hospedeiros de amplificação e mosquitos *Aedes*, transmitem os vírus, como já bem documentado para dengue (DENV), Zika (ZIKV) e chikungunya (CHIKV) (YOUNG, 2018).

O primeiro caso de dengue no Brasil (sorotipos DENV-1 e DENV-4) foi registrado em 1981 em Boa Vista, Roraima (ARAÚJO *et al.*, 2015) mas as ações de controle vetorial começaram na década de 1940, com foco na erradicação de *Ae. aegypti* para controle da Febre Amarela (YFV) (LÖWY, 1999). Em 1955 com advento do DDT - Dicloro-Difenil-Tricloroetano, e o esforço conjunto da Organização Pan-Americana da Saúde (OPAS) e a Organização Mundial da Saúde (OMS) *Ae. aegypti* foi considerado erradicado do Brasil (MAGALHÃES, 2016; BRAGA; VALLE, 2007; CONSOLI; OLIVEIRA, 1994).

Em 1960 *Ae. aegypti* foi reintroduzido e em 1973, foi mais uma vez considerado erradicado do país. No entanto, em 1976, a espécie foi reintroduzida em razão da urbanização acelerada e falhas na vigilância, o que resultou no primeiro surto de dengue em Boa Vista (1981). Em 1986 ocorreu a primeira epidemia de DENV-1 no Rio de Janeiro e com a introdução do DENV-2 em 1990, e DENV-3 em 2000, novos surtos se espalharam pelo país, o que levou a criação do Plano Nacional de Controle da Dengue (PNCD) (BRAGA; VALLE, 2007). O PNCD buscava reduzir a infestação de *Ae. aegypti* e diminuir a letalidade da dengue, através da integração de ações de vigilância epidemiológica e saneamento, educação em saúde, comunicação e mobilização social (MINISTÉRIO DA SAÚDE, 2001). Contudo, em 2010, com o ressurgimento de DENV-4, foram registrados 981.276 casos de dengue e em 2012 aproximadamente 4,5 milhões de casos de dengue foram notificados (ARAÚJO *et al.*, 2015)

O Brasil reporta mais casos de dengue do que qualquer outro país do mundo (PAHO/WHO, 2024). Esse elevado número de casos é resultado do aumento na distribuição e densidade dos vetores *Ae. aegypti* e *Aedes albopictus* e à circulação simultânea de diferentes linhagens e sorotipos virais (DENV-1, DENV-2, DENV-3 e DENV-4) (ADELINO *et al.*, 2021; LETA *et al.*, 2018). O primeiro registro de *Ae. albopictus*, no Brasil, é de 1986 (FORATTINI, 1986). Embora experimentalmente seja vetor competente para DENV-1, DENV-2, DENV-3, DENV-4 e ZIKV (FERREIRA-DE-LIMA *et al.*, 2020; GARCIA-REJON *et al.*, 2021), registros de infecção natural por esses arbovírus em populações brasileiras do mosquito são limitados. Com exceção para 1993 quando larvas de *Ae. albopictus* foram encontradas infectadas com DENV-1 (SERUFO *et al.*, 1993) e 2019 quando fêmeas adultas foram detectadas com DENV-1 e ZIKV no Espírito Santo (REZENDE *et al.*, 2020)

A ocorrência de dengue e outras arboviroses está diretamente relacionada aos padrões de abundância dos vetores (CARLSON; DOUGHERTY; GETZ, 2016; FREEMAN *et al.*, 2022; LETA *et al.*, 2018; RODRIGUEZ-FIGUEROA *et al.*, 1995). Nesse contexto, a heterogeneidade da paisagem urbana é um importante impulsionador para proliferação — ou redução — das populações desses mosquitos (HEMME *et al.*, 2010; WILKE *et al.*, 2019). Mosquitos adultos de *A. aegypti* percorrem distâncias relativamente curtas de ~100m, embora estimativas de dispersão mais longas (~800m) ou mais curtas (~30m) também tenham sido observadas (BROWN *et al.*, 2017; HARRINGTON *et al.*, 2005; MCDONALD, 1977). Nesse cenário, a cobertura vegetal e/ou fragmentos isolados de vegetação criam uma distribuição descontínua de potenciais criadouros e disponibilidade de hospedeiros humanos, influenciando diretamente a capacidade vetorial (BROWN *et al.*, 2017; MAFFEY *et al.*, 2022).

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Montane urban forests play a key role in mitigating arbovirus transmission

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ABSTRACT

The mosquitoes *Aedes aegypti* (Linnaeus, 1762) and *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae), the primary vectors of urban arboviruses, are invasive and synanthropic species with distinct habitat preferences. Their oviposition success is influenced by the degree of urban vegetation, shaping their distribution across different landscapes. However, the influence of urban forests on the distribution of these species, as well as the effects of altitude and associated climatic variations, remains poorly understood. To monitor their distribution across different urban biotopes, ovitraps were installed during the 2022 rainy season in three distinct environments in Ouro Preto and Mariana, Minas Gerais, Brazil: urban forest, landscaped residential area, and highly urbanized area. This sampling design replicated part of a study conducted a decade earlier in these cities, where these species likely established due to rising temperatures in the early 21st century.

Our results indicate that *Aedes* vector distribution and abundance are influenced by biotope type, city, and year ($p < 0.001$). *Ae. aegypti* was the most abundant species, particularly in urbanized areas, while *Ae. albopictus* exhibited a more homogeneous distribution across the urban landscape. Between 2012 and 2022, *Ae. aegypti* increased by 1189.7%, with the highest growth in Ouro Preto (74,300.0%). *Ae. albopictus* maintained overall stable abundance (+5.5%), with a 14,700.0% increase in Ouro Preto and a 31.5% decrease in Mariana. These findings suggest that well-managed urban green spaces may be essential for mitigating the risk of arbovirus transmission, including dengue, Zika, and chikungunya.

Keywords: *Mosquitoes, Insect vectors, Tropical diseases, Global warming, Urban ecology*.

Introduction

Aedes aegypti (Linnaeus, 1762) (Diptera: Culicidae) is probably one of the most adaptable and widespread mosquito species in the world (Tabachnick, 1991; Lounibos, 2002; Brown et al., 2011; Kraemer et al., 2015). Studies suggest that this monophyletic species may have originated in Africa approximately 17,000-25,000 years ago, where the ancestral form was generalist and zoophilic, breeding in tree holes (Brown et al., 2014; Gloria-Soria et al., 2016; Kotsakiozi et al., 2018; Soghigian et al., 2020). Three subspecies are currently known: *Ae. aegypti formosus*, a wild subspecies ecologically similar to the ancestral form found in eastern and southern sub-Saharan Africa; *Ae. aegypti queenslandensis*, originally described in Queensland, Australia, but found throughout Asia, Oceania, Europe, and North Africa; and the "domestic" form, *Aedes aegypti aegypti*, which has evolved to specialize in feeding on human blood and breeding in anthropogenic sites (Brown et al., 2014; McBride et al., 2014; Santos et al., 2022; Rose et al., 2023). Over the last 400-500 years, this form has colonized much of the tropical and subtropical continents, driven by human dispersal and trade routes (Gloria-Soria et al., 2016; Kotsakiozi et al., 2018).

The life history of *Ae. aegypti* has been strongly influenced by human behavior. The hypothesis better accepted to explain its speciation into the "domestic" form, predicted that *Ae. aegypti aegypti* evolved in West Africa, where human settlements originally formed adjacent to forests (Gloria-Soria et al., 2016). However, during prolonged annual droughts related to a drier period and the expansion of Sahara desert, when natural breeding sites for *Ae. aegypti* dried up, the human habit of storing water created a free and stable aquatic niche that facilitated the mosquitoes' adaptation to these new environments (Powell et al., 2018; Rose et al., 2020). Female mosquitoes would have liable breeding sites for oviposition around human settlements, even during the most critical period for species survival (Shiau et al., 2024), probably had little choice but to deposit their eggs in human-made water containers, creating a fidelity to human-made habitats (Powell et al., 2018). In addition to increasing number of favorable breeding sites, human settlements directed the feeding habits of mosquitoes toward the most abundant food resource, the humans themselves (Stensmyr, 2020). Thus, the intensity of the dry season, the scarcity of natural breeding sites, and the availability of alternative hosts can explain the speciation and high anthropophilia observed in *Ae. aegypti aegypti*, which is now the main vector of urban arboviruses worldwide (Rose et al., 2020; Rose et al., 2023; PAHO/WHO, 2024).

Arboviruses are currently the greatest challenge to health systems (PAHO/WHO, 2024). There are an estimated 537 viruses transmitted by arthropod vectors such as mosquitoes, ticks, and sandflies, of which more than 130 are known to cause disease ranging from mild to fatal in humans (Gyawali and Taylor-Robinson, 2017). Viruses ingested by mosquitoes from infected hosts are transmitted to new hosts during blood feeding after an extrinsic incubation period (Weaver and Reisen, 2010; Young, 2018). Although most arboviruses are predominantly zoonotic, the mechanism of transition from the enzootic cycle to a predominantly human cycle, in which humans serve as amplification hosts, and anthropophilic mosquitoes transmit the viruses, is well documented for dengue (DENV), zika (ZIKV), and chikungunya (CHIKV), the most important urban arboviruses today, transmitted by the invasive species *Ae. aegypti aegypti* and *Aedes albopictus* (Weaver and Reisen, 2010; Weaver et al., 2017; Young, 2018).

It is estimated that more than 80% of the world's population is vulnerable to insect-borne arboviruses (PAHO/WHO, 2024). This vulnerability is the result of anthropogenic changes that bring vectors, parasites, and hosts together in the same habitat (Wilke et al., 2021; Burkett-Cadena et al., 2022; Campos et al., 2023). Deforestation, rapid and unplanned urbanization, and socio-environmental characteristics such as poverty and lack of investment in public policies for education and socio-economic development, are synergistic drivers of arbovirus transmission in urban areas (Gregianini et al., 2017; Francisco et al., 2021; Rocha et al., 2023). In addition to such impacts, climate changes have created ideal temperatures for mosquitoes to thrive in tropical high altitudes (Pedrosa et al. 2020) and sub-tropical and non-tropical latitudes (Ryan et al., 2019).

Forests with high biodiversity and a greater number of competitors and predators tend to inhibit the presence of exotic and anthropophilic mosquitoes (Cunha et al., 2021). However, urbanization leads to the destruction of these habitats and changes the availability of resources (Duval et al., 2023). On the other hand, mosquito species specialized in anthropogenic environments, such as *Ae. aegypti aegypti*, thrive under conditions of deforestation, increased human population densities, and heterogeneity of urban infrastructure, especially in developing countries (Siqueira et al., 2022).

Given the importance of understanding how *Aedes* mosquito dynamics are affected by urbanization, our study is based on the hypothesis that urban forests play a central role the distribution of *Ae. aegypti* and *Ae. albopictus*, along with altitude and consequential climate differences. We explored the presence and abundance of *Ae. aegypti* and *Ae. albopictus* in a gradient of urban construction density, from urban parks towards densely pavemented centers, with neighbourhoods with gardened neighbourhoods between these extremes, over a 10-year interval (2012 and 2022). The study was conducted in two municipalities in Minas Gerais, Brazil: Mariana and Ouro Preto.

Ouro Preto is a high-altitude town, around 1,500 meters above sea level, where *Ae. aegypti* and *Ae. albopictus* invaded only recently, most likely due to warmer climates in the last 15-20 years (Pedrosa et al., 2020). The first sustainable populations of these mosquitos were documented in 2009 and first endemically transmitted cases of dengue in 2012 (Pedrosa et al., 2020). Mariana is a high to mid-altitude town and had higher densities of these mosquito species than Ouro Preto around 2012 (Predosa et al. 2020). Our prediction is that more forested areas will favor *Ae. albopictus* presence and abundance, while the opposite, *Ae. aegypti*. We also expect that, due to differences in local temperatures, number of individuals of both species might have been kept consistently higher in Mariana than Ouro Preto.

Materials and Methods

Study Area

Mariana (20°22′40″S and 43°24′57″W, at an elevation of 750 meters above sea level, hereafter asl) and Ouro Preto (20°23′08″S and 43°30′29″W, at 1500 meters asl) are high to mid- and high altitude tropical municipalities in the state of Minas Gerais, Brazil. The two cities are approximately 12 km apart and are located about 100 km from the capital, Belo Horizonte. Mariana has an area of 1,194 km² and an estimated population of 61,387 inhabitants in 2022, with a population density of about 5140 inhabitants/km² (IBGE, 2022). The municipality's climate is classified as tropical highland, with average temperatures ranging from 18°C to 22°C and an average annual rainfall of 1,804 mm (Ozório, 2000; INMET).

Ouro Preto has an area of 1,245 km² and an estimated population of 74,821 inhabitants in 2022, with a population density of approximately 6006 inhabitants/km² (IBGE, 2022). The municipality's climate is classified as tropical highland, with average temperatures ranging from 17°C to 18.5°C and an average annual rainfall of 2,018 mm, with irregular distribution and rainfall, concentrated in the summer (Werneck et al., 2000; INMET).

In social terms, Mariana and Ouro Preto have a medium Human Development Index (0.742 and 0.741, respectively) and moderate income inequality (Gini coefficient of 0.5 for Mariana and 0.7 for Ouro Preto) (IBGE, 2022).

Sampling Design

We used a similar sampling design to the one applied by Pedrosa *et al.* (2020) in 2012, and adjusted sampling effort to compare the population sizes of mosquitoes 10 years after. Briefly, for each town, 90 ovitraps were placed on trees, shrubs, and other supports that allowed them to be suspended approximately 1.5 m above the ground in shaded areas, where they remained for three days. No attractants were used, to capture the female success in laying eggs in a most likely urban, eutrophic, microhabitat. Samples were repeated three times in each town between March and April 2022, for a total sampling effort of 540 traps.

Three different biotopes were used in each town. Biotope I corresponds to an urban, arboreous, green space. In Mariana, this area had about 20 hectares, while in Ouro Preto it had about 14 hectares. Biotope II, an intermediate area, located nearby the green areas, with low density of gardened houses, some patches of natural vegetation and high permeable soil ratio between the houses. Biotope III was an intensely built neighbourhood with high impermeability, located about 500 meters from the studied green area or any other significant green area.

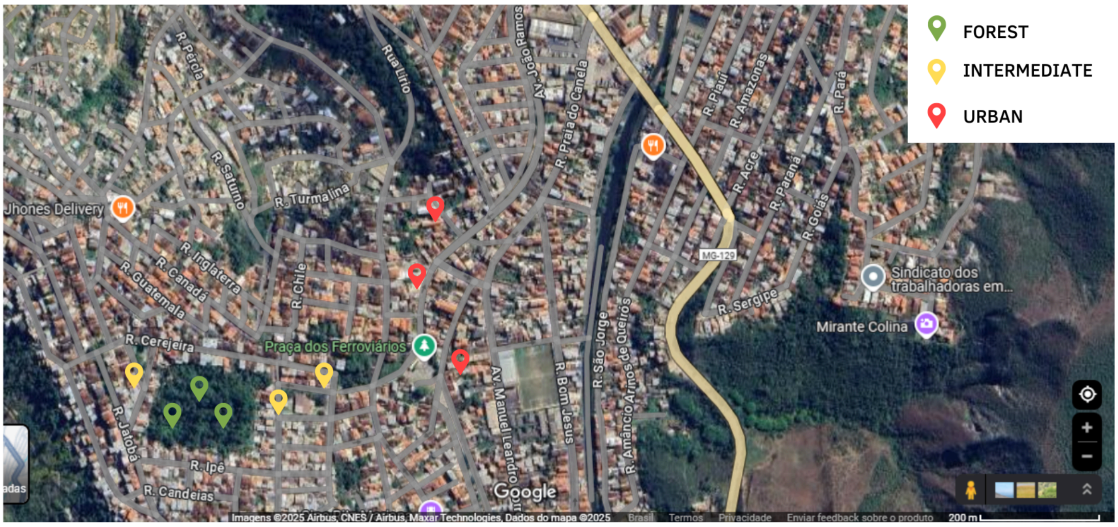

Figure 1 – Map showing the distribution of ovitraps in different urban biotopes in Mariana, Minas Gerais, Brazil. Green markers represent ovitraps placed in urban forest areas, yellow markers indicate traps in residential areas with gardens, and red markers correspond to traps in highly urbanized areas.

The background satellite image is from Google Maps, with street names and landmarks for spatial reference.

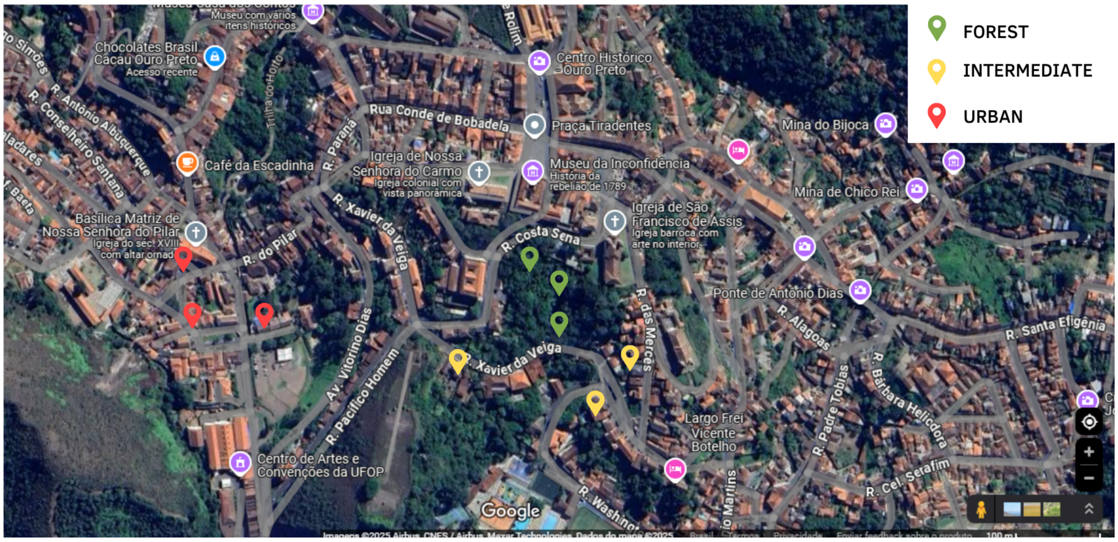

Figure 2 – Map showing the distribution of ovitraps in different urban biotopes in Ouro Preto, Minas Gerais, Brazil. Green markers represent ovitraps placed in urban forest areas, yellow markers indicate traps in residential areas with gardens, and red markers correspond to traps in highly urbanized areas. The background satellite image is from Google Maps, with street names and landmarks for spatial reference.

Temporal Analysis

In 2012, Pedrosa et al. (2020) installed 180 traps in both Mariana and Ouro Preto, divided between forest, intermediately urbanized, and intensively urbanized biotopes (hereafter referred to as forest, intermediate, and urban). In 2022, we increased the sampling effort to 540 traps to improve the precision of the estimates and the statistical power of comparisons between biotopes and cities. The methodology and biotopes assessed remained the same from the original study, ensuring valid comparisons between the two periods.

Statistical Analysis

To investigate the effects of town (Mariana and Ouro Preto) and biotope type (forest, intermediate, and urban) on *Aedes* abundance, we used the Kruskal-Wallis test with Bonferroni correction and a negative binomial generalized linear model (GLM). The data structure was analyzed to identify potential multicollinearity problems among the predictor variables (biotope, town, mosquito species, and year) using the variance inflation factor (VIF). The GLM was used to assess changes in *Aedes* abundance and distribution between 2012 and 2022. All analyses were performed using R software version 4.4.2, which allowed us to assess the main effects of each factor and the interactions between towns and biotope types on vector abundance.

Results

In 2022, a total of 4,149 Aedini eggs were collected in the municipalities of Mariana (2,386 eggs) and Ouro Preto (1,763 eggs). From these, 2,289 eggs successfully hatched into adult mosquitoes: 1,869 *Aedes aegypti* and 420 *Aedes albopictus*. When comparing the two municipalities, Mariana had a higher overall abundance of *Aedes* mosquitoes, with 1,386 adults compared to 903 adults in Ouro Preto. This pattern was consistent for both species, with *Ae. aegypti* being more abundant in Mariana (1,132 individuals) than in Ouro Preto (737 individuals), and *Ae. albopictus* also showing higher numbers in Mariana (254 individuals) than in Ouro Preto (166 individuals).

In both municipalities, intermediate and urban biotopes, characterized by higher building density and soil impermeability, showed the highest abundance of *Aedes* mosquitoes, with 613 and 1,110 individuals, respectively. In these biotopes, *Ae. aegypti* was the dominant species with 1,473 individuals (Figure 3), while *Ae. albopictus* accounted for 250 individuals (Figure 4). In the forest biotopes, 566 adult *Aedes* mosquitoes were recorded, of which 396 were *Ae. aegypti*.

We observed significantly higher *Ae. aegypti* abundance in Mariana compared to Ouro Preto (Kruskal-Wallis: $\chi^2 = 22.466$, df = 1, $p < 0.001$; Dunn: p.adj < 0.001), while *Ae. albopictus* showed no significant difference between cities (Kruskal-Wallis: $\chi^2 = 2.747$, df = 1, $p = 0.097$; Dunn: p.adj = 0.097). Regarding biotopes, *Ae. aegypti* abundance was significantly higher in urban areas compared to forest (Dunn: p.adj < 0.001) and intermediate biotopes (Dunn: p.adj = 0.0005) (Kruskal-Wallis: $\chi^2 = 32.543$, df = 2, $p < 0.001$) (Figure 3). No significant difference was observed between forest and intermediate biotopes (Dunn: p.adj > 0.05). In contrast, *Ae. albopictus* showed a homogeneous distribution across the three biotopes (Kruskal-Wallis: $\chi^2 = 1.777$, df = 2, $p = 0.411$; Dunn: $p.adj > 0.05$) (Figure 4).

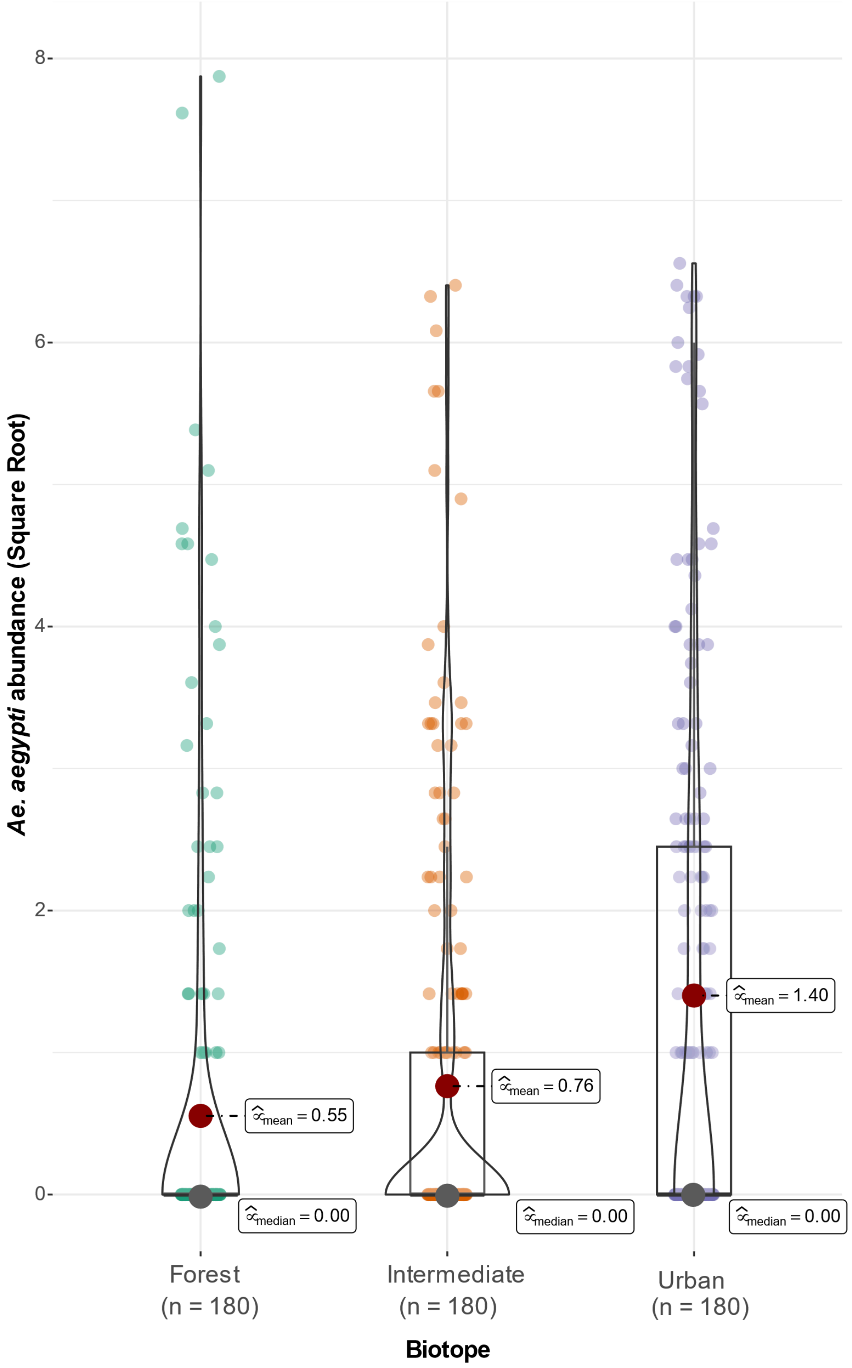

Figure 3 – Abundance of *Aedes aegypti* mosquitoes in the studied biotopes in 2022 (Kruskal-Wallis: $\chi^2 = 32.543$, df = 2, $p < 0.001$).

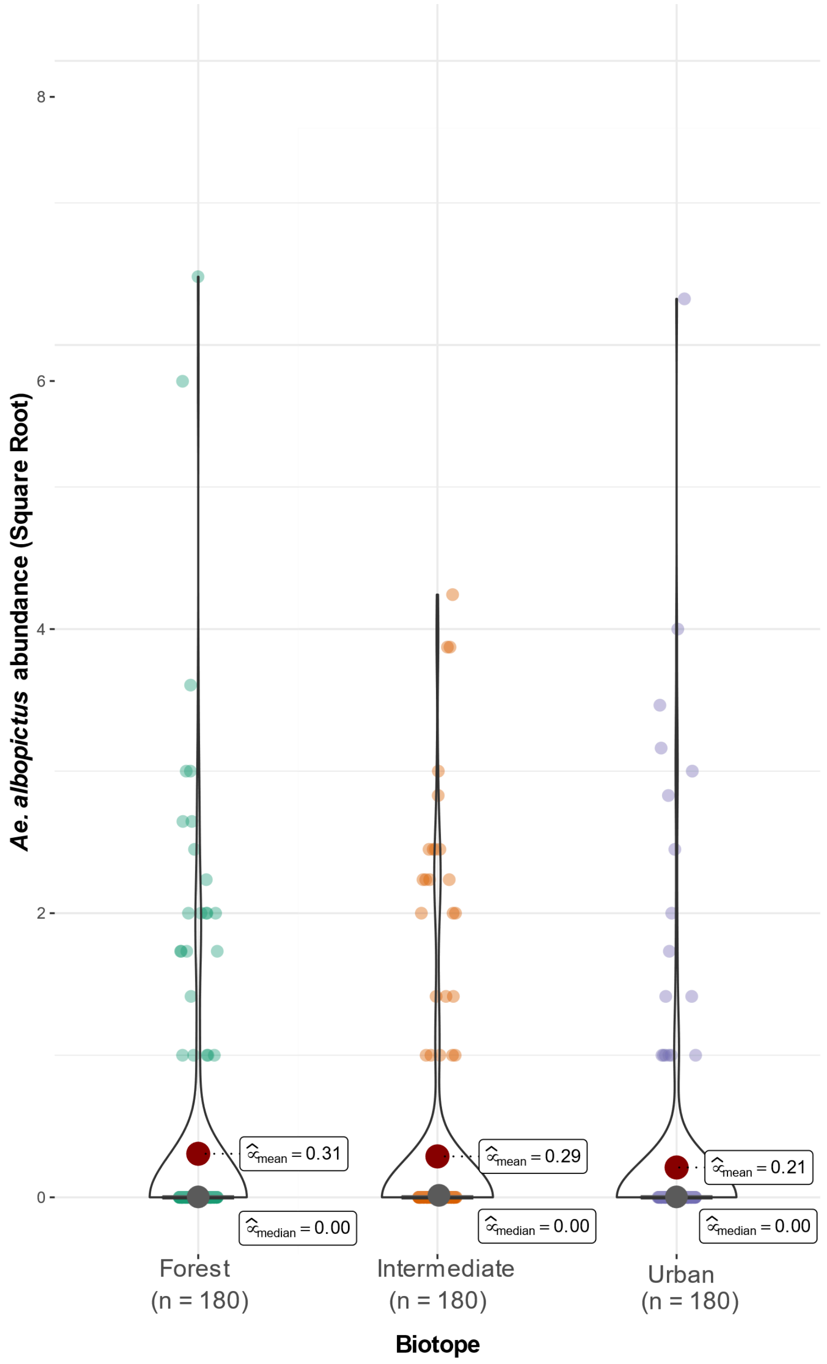

Figure 4 – Abundance of *Aedes albopictus* mosquitoes in the studied biotopes in 2022 (Kruskal-Wallis: $\chi^2 = 1.777$, df = 2, $p = 0.411$; Dunn: p.adj > 0.05).

Temporal analysis revealed a significant increase in *Aedes aegypti* abundance in 2022 compared to 2012 (+1189.7%), especially in urban biotopes (ANOVA: $\chi^2 = 32.693$, df = 1, $p < 0.001$) (Figure 7). However, this expansion varied considerably between cities: in Mariana (Figure 8), *Ae. aegypti* abundance increased by ~681%, while in Ouro Preto, the increase was even more pronounced, reaching 743 times higher abundance in 2022 compared to 2012 (Figure 9).

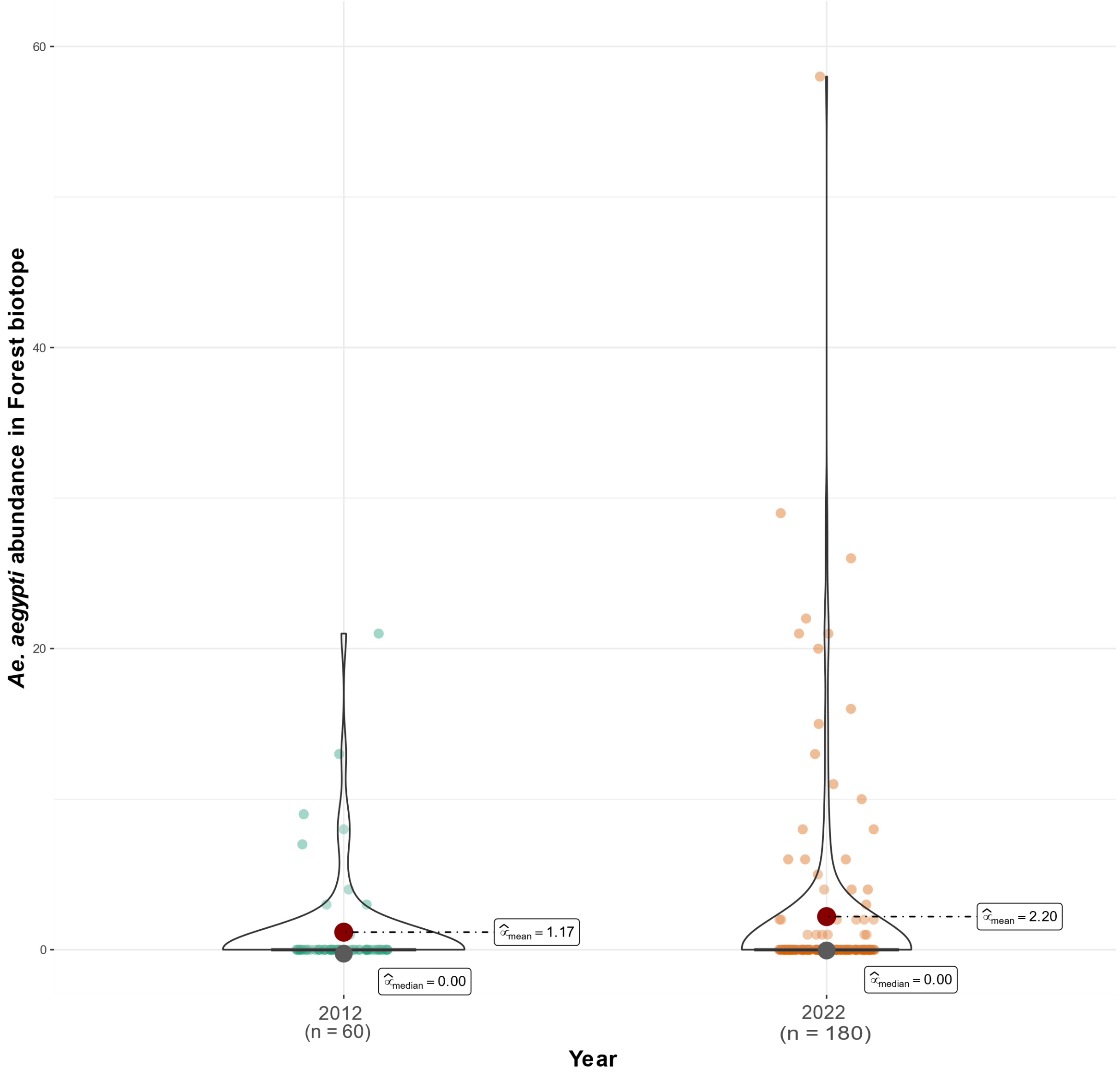

Figure 5 – Abundance of *Aedes aegypti* in 2012 and 2022 in Forest biotope.

(W?$_a$□□-$_6$?$_i$□□$_e$y = 5237.00, p = 0.61, r$_{\beta i}$□$_{eria}$□ $_{ra}$□□ = –0.03, CI$_{95}$% [–0.20, 0.14], n$_{o\beta}$□ = 240).

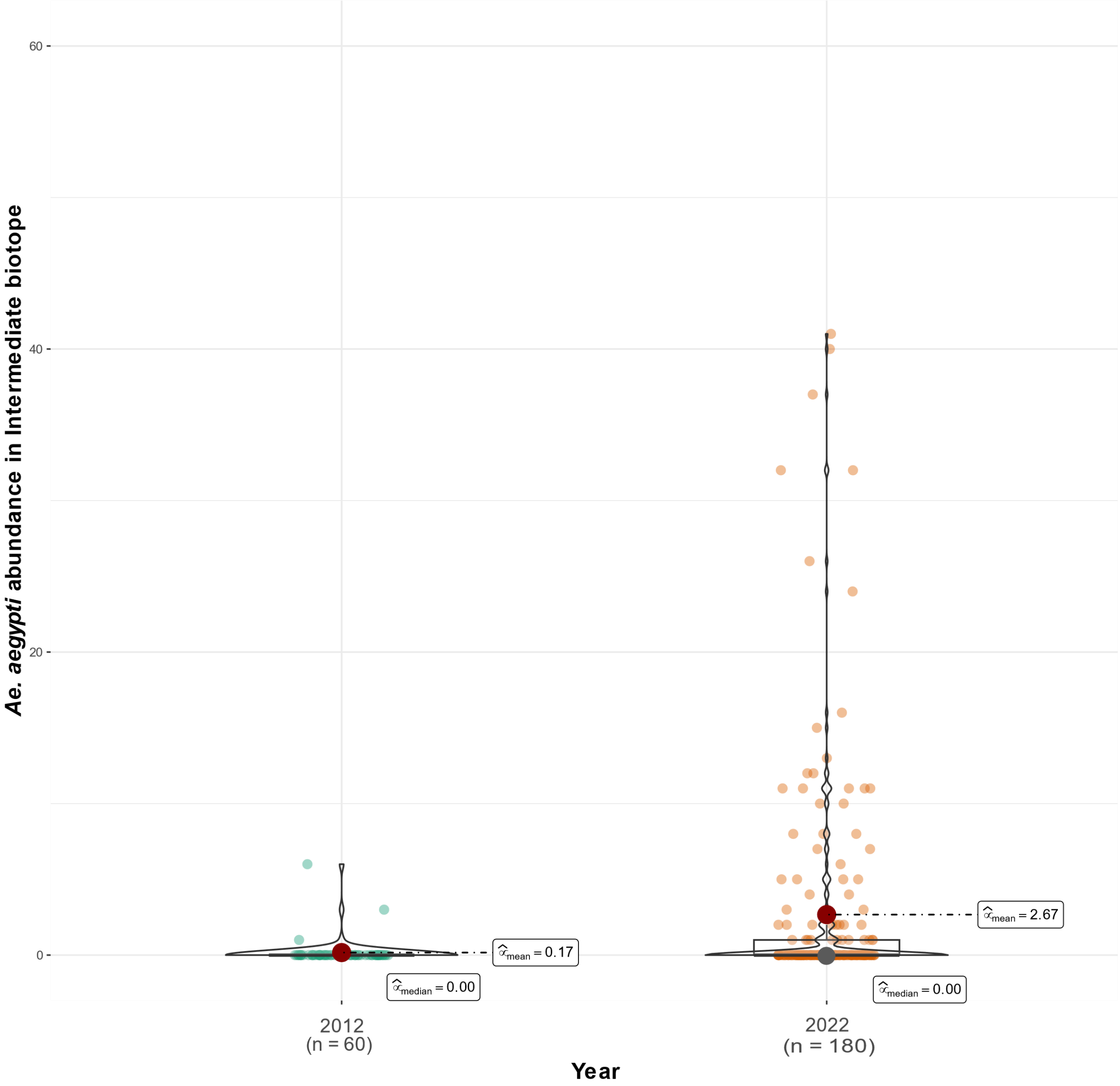

Figure 6 – Abundance of *Aedes aegypti* in 2012 and 2022 in Intermediate biotope.

(W$_{a}$□□-$_{6}$i□□$_{e}$y = 4056.00, p = 9.91e–05, r$_{βi}$□$_{eria}$□ $_{ra}$□□ = –0.25, CI$_{95}$% [–0.40, –0.08], n$_{oβ}$□ = 240).

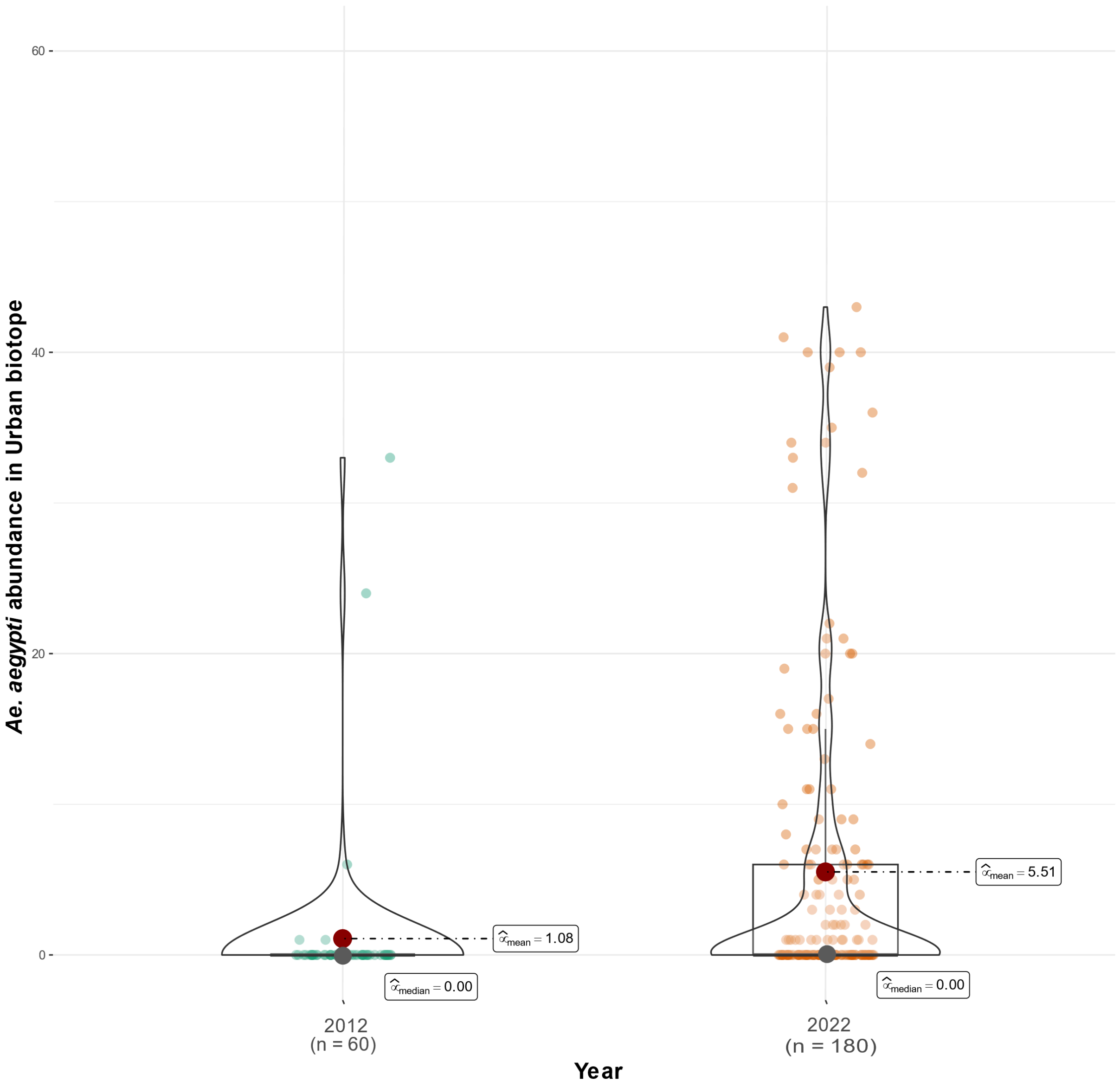

Figure 7 – Abundance of *Aedes aegypti* in 2012 and 2022 in Urban biotope.

(W▮$_a$□□-$_6$▮$_i$□□$_e$y = 3317.00, p = 2.45e–07, r$_{\beta i}$□$_{eria}$□ $_{ra}$□□ = –0.39, CI$_{95}$% [–0.52, –0.23], n$_{o\beta}$□ = 240).

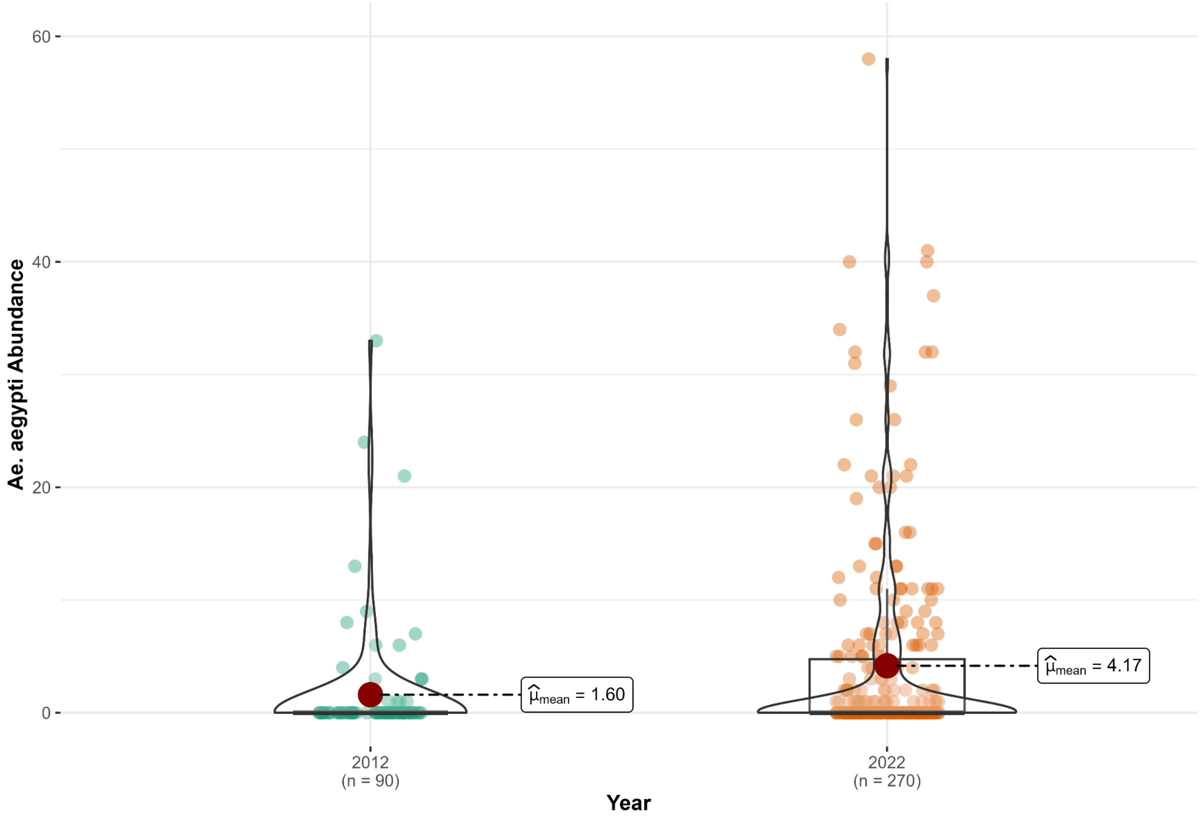

Figure 8 – Abundance of *Aedes aegypti* in 2012 and 2022 in Mariana.

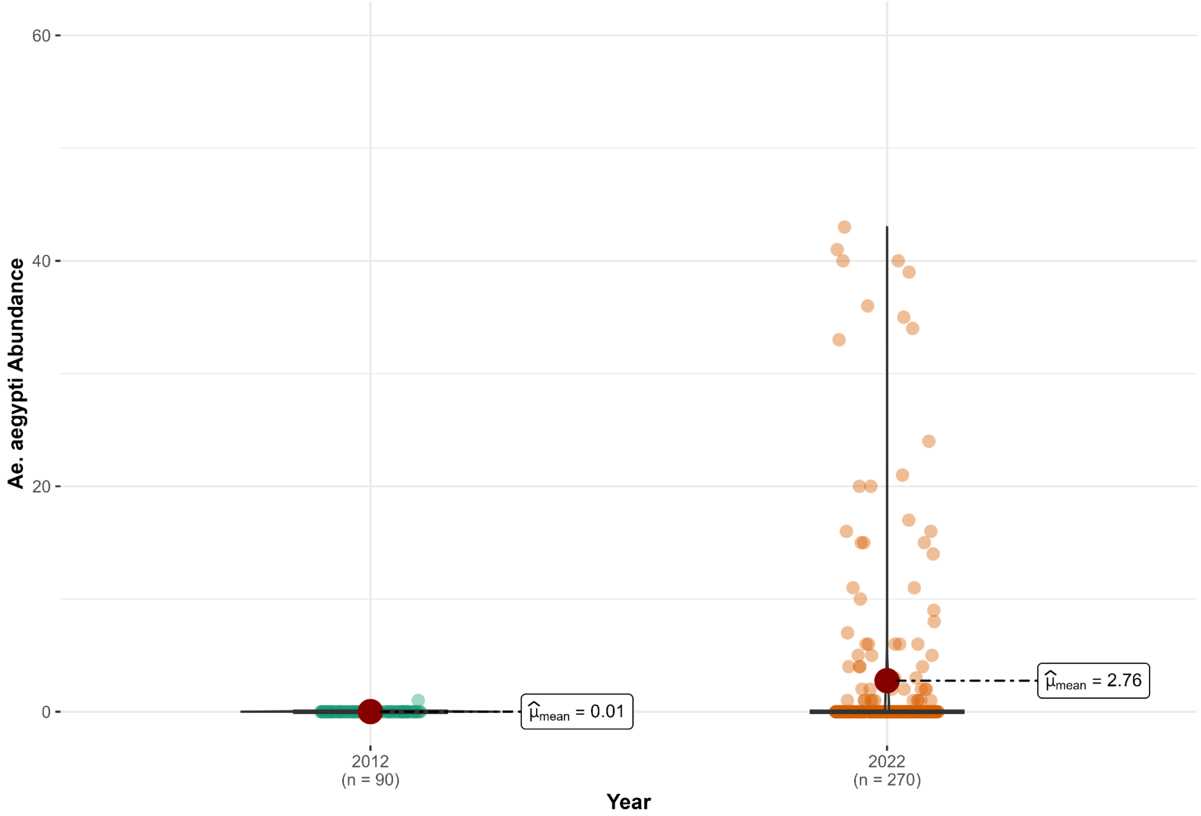

Figure 8 – Abundance of *Aedes aegypti* in 2012 and 2022 in Ouro Preto.

For *Ae. albopictus*, the effect of biotope was not statistically significant (ANOVA: $\chi^2 = 0.947$, df = 2, $p > 0.05$) (Figures 9, 10 and 11). However, *Ae. albopictus* showed distinct trends between cities. Although no significant overall temporal variation was detected (ANOVA: $\chi^2 = 0.186$, df = 1, $p = 0.666$), its abundance decreased by approximately 31% in Mariana (Figure 12). In contrast, a striking increase was observed in Ouro Preto, where its abundance was ~147 times higher in 2022 than in 2012, despite starting from very low values (Figure 13). These results indicate that 2022 was positively associated with an increase in *Ae. aegypti* abundance and a relative decrease in *Ae. albopictus*, with opposite trends in the two cities.

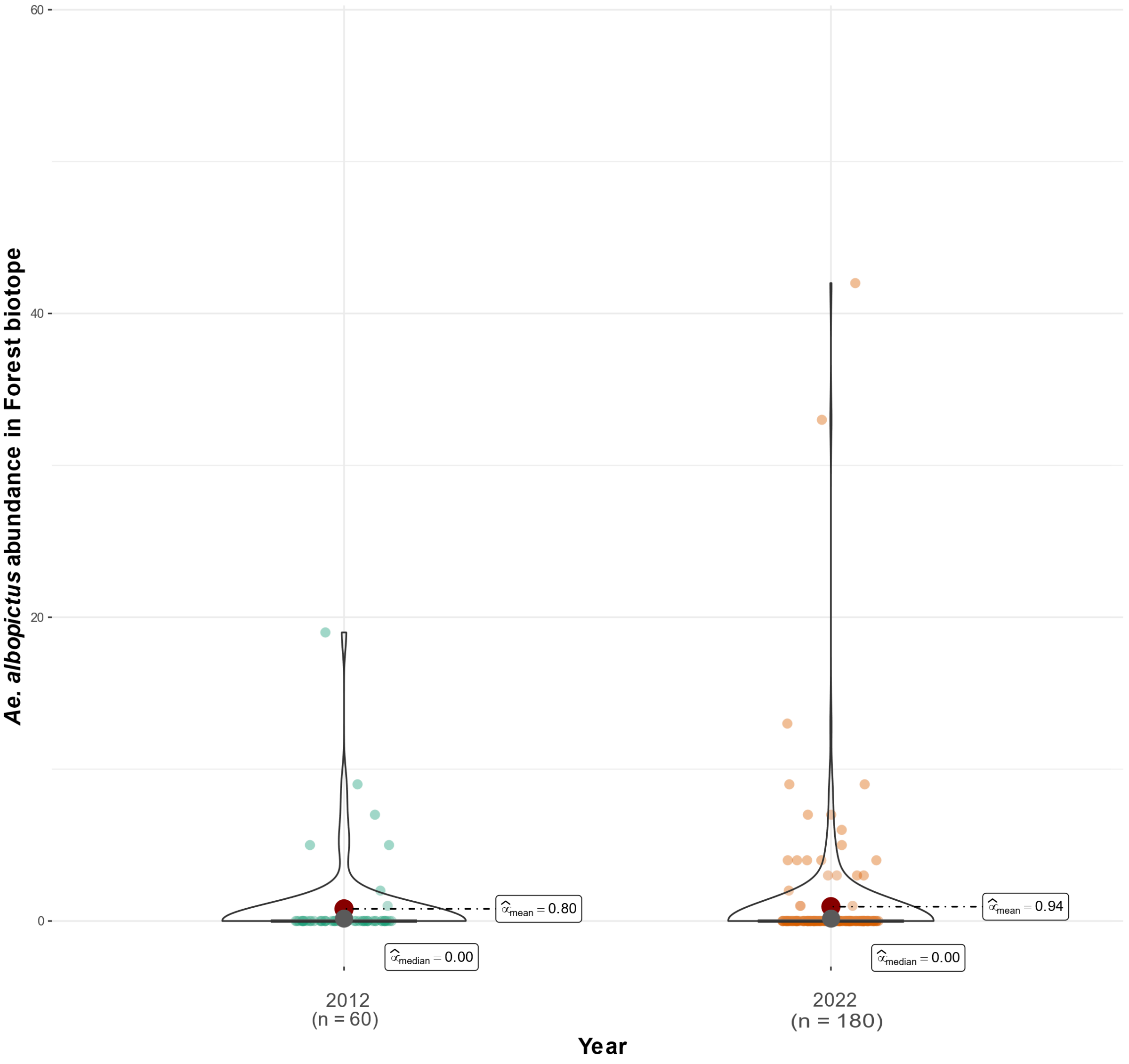

Figure 9 – Abundance of *Aedes albopictus* in 2012 and 2022 in Forest biotope

(W$_{\text{a□□-}}$$_{\text{б}}$$_{\text{i□□}}$$_{\text{e}}$y = 5325.00, p = 0.78, r$_{\text{βi□eria□ ra□□}}$ = 0.01, CI$_{95\%}$ [–0.18, 0.15], n$_{\text{oβ□}}$ = 240).

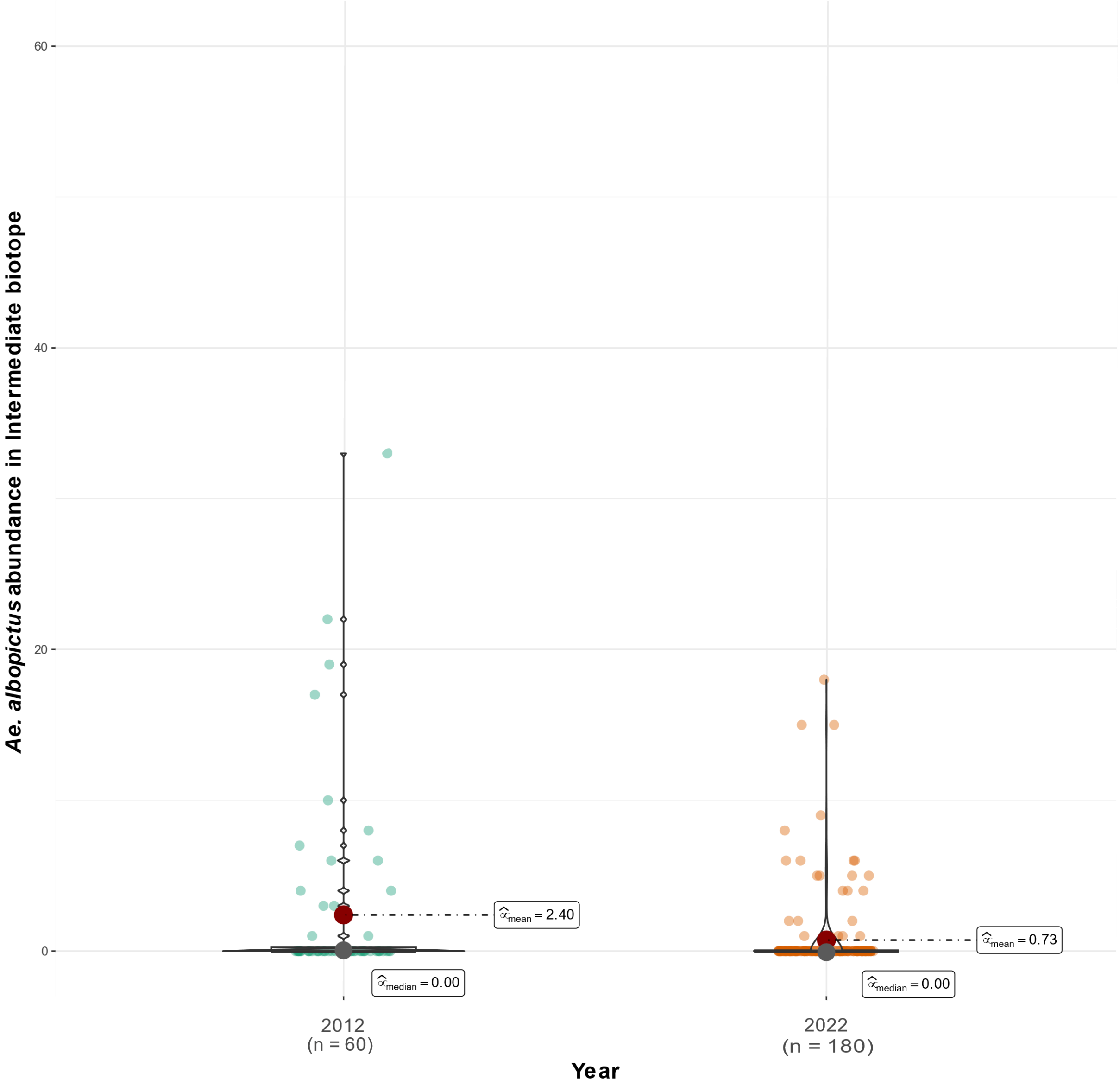

Figure 10 – Abundance of *Aedes albopictus* in 2012 and 2022 in Intermediate biotope

($W_{\text{Mann-Whitney}} = 6078.50$, $p = 0.02$, $\widehat{r}_{\text{biserial}}^{\text{rank}} = 0.13$, $\text{CI}_{95\%}$ [–0.04, 0.29], $n_{\text{obs}} = 240$).

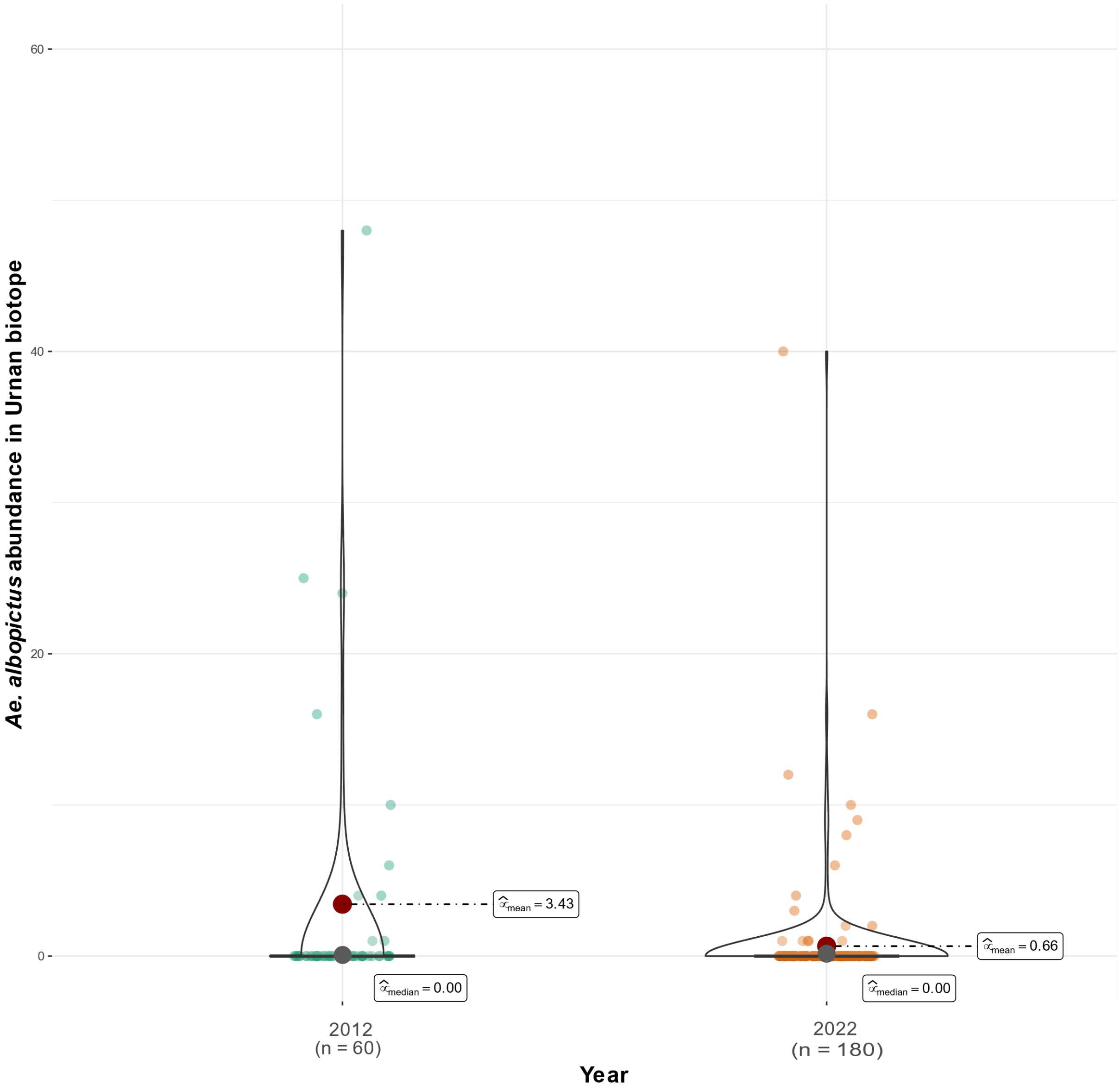

Figure 11 – Abundance of *Aedes albopictus* in 2012 and 2022 in Urban biotope

($W_{\text{Mann-Whitney}}$ = 5917.00, p = 0.05, $\hat{r}_{\text{biserial}}^{\text{rank}}$ = 0.10, $\text{CI}_{95\%}$ [−0.07, 0.26], n_{obs} = 240).

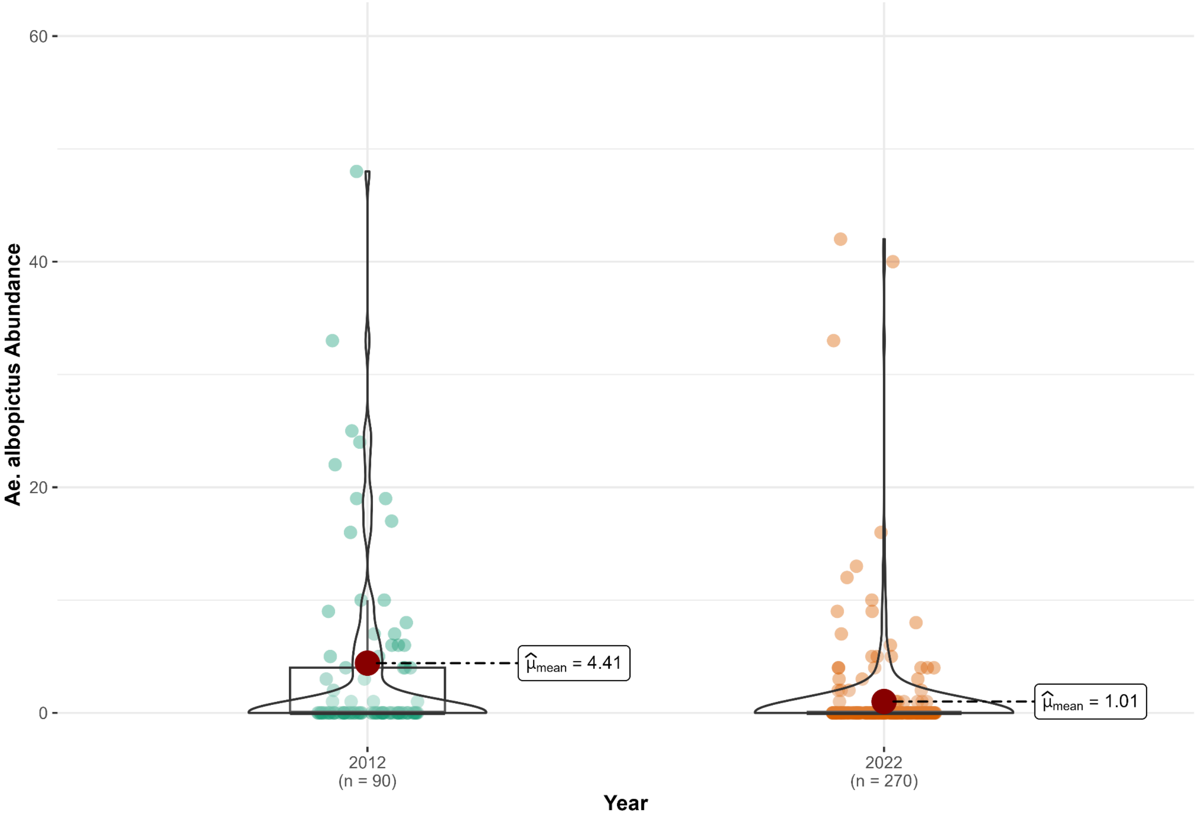

Figure 12 – Abundance of *Aedes albopictus* in 2012 and 2022 in Mariana.

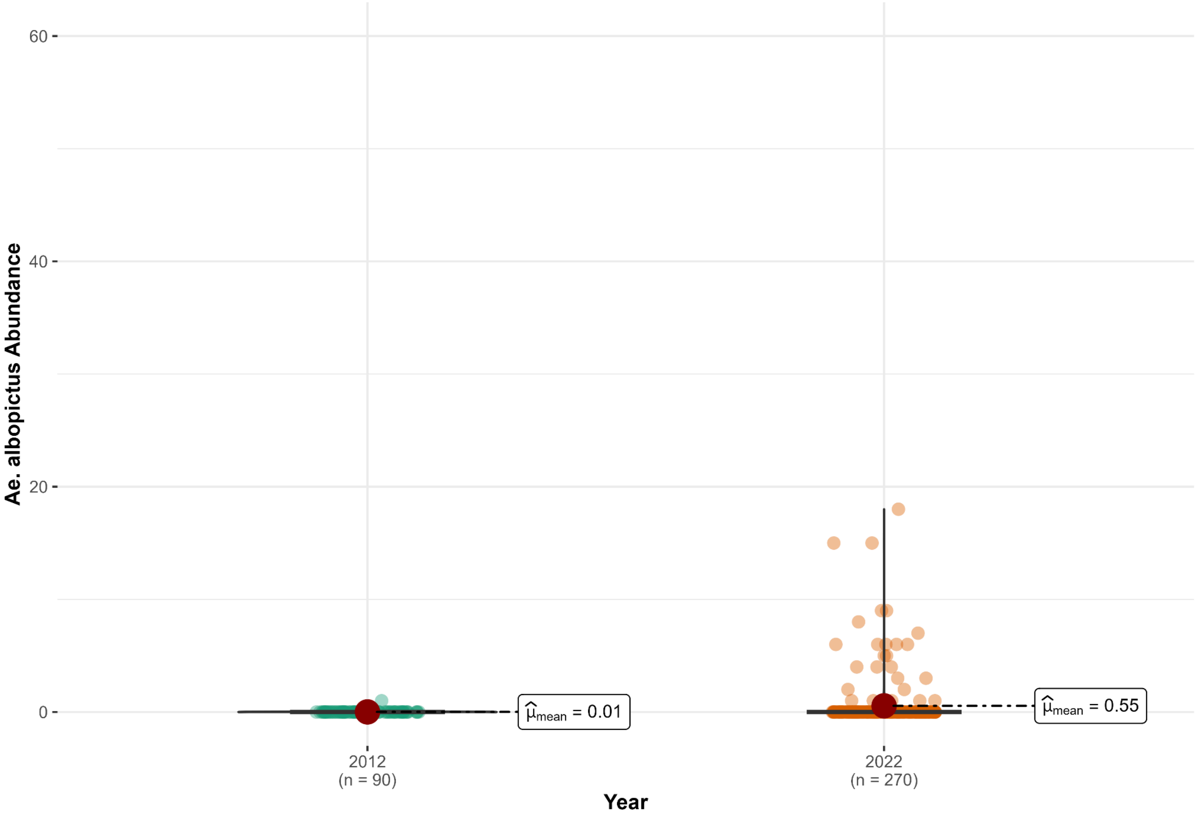

Figure 13 – Abundance of *Aedes albopictus* in 2012 and 2022 in Ouro Preto.

In addition, the abundance of both species was significantly lower in Ouro Preto than in Mariana (*Ae. aegypti*: ANOVA: $\chi^2 = 19.821$, df = 1, $p < 0.001$; *Ae. albopictus*: ANOVA: $\chi^2 = 23.180$, df = 1, $p < 0.001$). The model showed good performance, with well behaved residuals and adequate dispersion (*Ae. aegypti*: dispersion ratio = 0.565; *Ae. albopictus*: dispersion ratio = 0.322).

Discussion

Aedes aegypti (originating from Africa) and *Ae. albopictus* (originating from Asia) are the main invasive species involved in the transmission of urban arboviruses such as dengue, chikungunya, and zika (Kraemer et al., 2015; Leta et al., 2018). In the Americas, both species are distributed across the continent, with records of *Ae. aegypti* dating back approximately 300 years (Powell et al., 2018) and *Ae. albopictus* around 40 years (Garcia-Rejon et al., 2021). Our results confirm that urban environments with limited permeability and scarce vegetation fragments are positively associated with the distribution and abundance of both species, especially for *Ae. aegypti*. This observation is consistent with previous studies showing a negative association between dengue incidence and vegetation quantity (Araujo et al., 2015; Cao et al., 2017; Cunha et al., 2020), as well as the combined effect of vegetation quantity and quality (Meza-Ballesta & Gónima, 2014).

The occurrence of dengue and other arboviruses is directly related to vector abundance patterns (Carlson et al., 2016; Freeman et al., 2022; Leta et al., 2018; Rodriguez-Figueroa et al., 1995), which, as we observed here, are influenced by forest remnants. As highlighted by Pedrosa *et al.*, (2020), climate change, such as warmer winters, has facilitated the invasion of *Aedes aegypti* and *Aedes albopictus* in tropical mountainous environments. Our results demonstrate this invasion and establishment, as we observed that the years 2012 and 2022 are positively associated with an increase in the abundance of *Ae. aegypti* and a decrease in the relative abundance of *Ae. albopictus*.

Although *Ae. albopictus* is susceptible to infection and capable of transmitting most viruses tested experimentally — including eight *Alphavirus*, eight *Flavivirus*, and four *Bunyavirus*, three of the major arbovirus genera that include human pathogens (Paupy et al., 2009) — *Ae. aegypti* is still considered a more competent vector under natural conditions in South America. This competence is attributed to traits such as its higher survival rate at elevated temperatures (Mordecai et al., 2017) and strong anthropophilic and endophilic behavior (Lambrechts et al., 2010; Severson & Behura, 2016; Ferreira et al., 2023). Although *Ae. aegypti* showed a substantial increase in abundance, our models did not detect a significant interaction with *Ae. albopictus*, suggesting that their population dynamics are shaped primarily by independent environmental and anthropogenic factors rather than direct competition.

Resource availability at the microgeographic (neighborhood) scale appears to be an important factor in the expansion or decline of mosquito vector populations (Wilke et al., 2020). Species such as *Ae. aegypti*, *Ae. albopictus*, and *Culex quinquefasciatus* find ideal conditions to thrive in human-modified environments due to the absence of natural predators, the availability of artificial aquatic habitats, increased human population density, and higher temperatures due to climate change (Wilke et al., 2021). Rose et al. (2020) suggested that seasonal variations in rainfall and human density are fundamental to the convergence of *Ae. aegypti* and humans (Rose et al., 2020). These convergent factors favoring highly anthropophilic species, such as *Ae. aegypti* and *Cx. quinquefasciatus*, making them the primary vectors of arboviruses such as chikungunya, dengue, yellow fever (YFV), West Nile virus (WNV), and Zika (Wilke et al., 2019; Cunha et al., 2021; Silva et al., 2024).

Another determinant of the spread of urban arboviruses, particularly in cities of developing countries, is socioenvironmental characteristics (Lorenz, Castro, et al., 2020; Francisco et al., 2021). Studies show that socioeconomic vulnerability is strongly associated with a higher incidence of vector-borne diseases due to inadequate housing and sanitation conditions that promote mosquito proliferation (Lorenz, Castro, et al., 2020; Lorenz, Chiaravalloti-Neto, et al., 2020). Poverty and lack of investment in public policies for education and socioeconomic development have been linked to the heterogeneous distribution of dengue cases within cities (Zellweger et al., 2017). Furthermore, low-income urban and peri-urban areas often have less vegetation due to dense urbanization, lack of proper urban planning, and irregular land occupation with non-forest open areas prone to accumulate refuse, which become oviposition hotspots (Zellweger et al., 2017; Siqueira et al., 2022). All these features favor urbanized vector species with large populations that are difficult to control.

The maintenance of urban forests and the establishment of protected areas play a fundamental role in the conservation of biodiversity, a resource of great importance for human health (Keesing & Ostfeld, 2021). Urbanization is strongly correlated with a reduction in the abundance and richness of native species, while promoting the spread of exotic species and potential hosts for zoonotic pathogens (Wilke et al., 202; Medeiros-Sousa et al. 2013). The abundance of *Aedes* species in urban centers is shaped by landscape elements (Lorenz, Castro, et al., 2020), and areas with less vegetation have a greater availability of potential breeding sites for these domesticated mosquitoes, thus influencing the occurrence of arboviruses through increased vector-host contact (Cunha et al., 2021; Siqueira et al., 2022). Our findings highlight the importance of urban landscape characteristics, particularly green space availability, in regulating *Aedes* mosquito populations and shaping arbovirus transmission risk. These results reinforce the need to integrate environmental management and urban planning strategies into vector control programs to mitigate the impact of mosquito-borne diseases.

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