UNIVERSITY OF SAO PAULO
INSTITUTE OF TROPICAL MEDICINE

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Environmental and social factors and its relation with *Aedes aegypti* demographics

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Fatores sociais e ambientais e suas relações com a demografia do mosquito *Aedes aegypti*

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Dissertation presented to Faculdade de Medicina of the Universidade de São Paulo, in order to obtain the Master of Science (MSc.) degree

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Dedications

Dedico este trabalho para todos que, assim como eu, acreditam que a valorização da Ciência é um dos caminhos para um futuro melhor. Espero estarmos vivos para vermos este futuro.

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Espero ter todos vocês por muitos e muitos outros anos em minha vida.

Resumo

Ramos Santos K. Fatores sociais e ambientais e suas relações com a demografia do mosquito Aedes aegypti [dissertação]. Sao Paulo: Faculdade de Medicina, Universidade de São Paulol; 2024.

Aedes aegypti é um mosquito urbano, pantropical e antropofílico. É um dos mais importantes vetores de patógenos do mundo, entre eles os vírus Dengue, Zika e Chikungunya. Sua alta variabilidade genética propicia fácil adaptação a diferentes ambientes, fazendo com que a sua mitigação seja um desafio complexo. Estima-se que diversas condições ambientais e sociais influenciem sua disseminação. Entretanto, poucas dessas condições foram testadas considerando sua relação com dados epidemiológicos. Neste cenário, o município de Santos é uma adequada cidade-modelo para estudo. Cidade portuária localizada no Estado de São Paulo, é altamente urbanizada, socialmente heterogênea, tem mosquitofauna monitorada por 480 armadilhas, e concentra numerosos casos de Dengue. O objetivo desta pesquisa foi a investigação da correlação entre fatores ambientais/sociais e a infestação do mosquito *Ae. aegypti* na região portuária de Santos. Para fins estatísticos, foram utilizados os 671 setores censitários (SC) contíguos (definidos pelo IBGE) da região insular da cidade, para os quais se conhece a vulnerabilidade social (VS) e densidade humana (DH). Dados de temperatura (TM) e pluviosidade (PL) foram registrados pela Defesa Civil da cidade. Dados de infestação por mosquitos *Ae. aegypti* (MO) foram semanalmente obtidos nas armadilhas pela prefeitura. Os softwares R e QGIS foram utilizados para o tratamento de dados e estatística. TM e PL mensais foram comparadas com a captura mensal de MO correspondentes ao mesmo período. DH e VS foram comparados de acordo com as semanas epidemiológicas. A cobertura vegetal (NDVI) da cidade foi estimada mediante imagens satelitais. Cerca de 85 mil fêmeas *Ae. aegypti* foram capturadas entre 2012-2022. MO variou sazonalmente e entre os anos, com maior pico durante a pandemia de SarsCov2. Houve correlação positiva entre MO e TM com um atraso de cerca de 9 semanas, sugerindoque o calor estimula a proliferação de mosquitos a médio prazo. Esse resultado é consistente com a literatura e pode indicar efeitos de ilhas de calor. Correlação positiva, porém mais fraca, surgiu entre MO e PL também com atraso de 9 semanas. Assim, TM parece ser um fator influente a todo o desenvolvimento do mosquito, enquanto PL tenha importância mais restrita à fase larval. Não houve correlação significativa entre MO e VS, o que é corroborado por estudos anteriores também em Santos. Não houve correlação entre MO e DH. Estudos anteriores também descrevem que *Ae. aegypti* possui baixa oviposição em áreas de maior DH que já foram urbanizadas, sendo HD um fator mais importante em locais de urbanização rápida e não organizada. Houve baixa correlação negativa entre NDVI e MO, indicando que cobertura vegetal tem papel antagonista, embora fraco, à proliferação do mosquitos. Em conjunto, esses achados auxiliam a definir quando e onde a mitigação do mosquito deve ser priorizada.

KEY-WORDS: Epidemiologia. Culicidae. Dengue. Saúde global. Ecossistema Urbano.

ABSTRACT

Ramos Santos K. Environmental and social factors and its relation with *Aedes aegypti* demographics [dissertation]. Faculdade de Medicina, Universidade de São Paulo; 2024.

Aedes aegypti is an urban, pantropical, and anthropophilic mosquito. It is one of the most important vectors of pathogens in the world, including Dengue, Zika, and Chikungunya viruses. Its high genetic variability allows it to easily adapt to different environments, making its mitigation a complex challenge. It is estimated that several environmental and social conditions influence its dissemination. However, few of these conditions have been tested considering their relationship with epidemiological data. In this scenario, the municipality of Santos is a suitable model city for study. A port city located in the State of São Paulo, it is highly urbanized, socially heterogeneous, has mosquito fauna monitored by 480 traps, and has numerous cases of Dengue. The objective of this research was to investigate the correlation between environmental/social factors and *Ae. aegypti* mosquito infestation in the port region of Santos. For statistical purposes, the island region of the city was divided by IBGE into 671 contiguous census sectors (SC), for which social vulnerability (SV) and human density (DH) are known. Temperature (TM) and rainfall (PL) data were recorded by the city's Civil Defense. Data on infestation by *Ae. aegypti* (MO) were collected weekly from traps by the city hall. The R and QGIS software were used for data processing and statistics. Monthly TM and PL were compared with the monthly MO capture corresponding to the same period. DH and SV were compared according to epidemiological weeks. The city's vegetation cover (NDVI) was estimated using satellite images. Around 85 thousand female *Ae. aegypti* were captured between 2012-2022. MO varied seasonally and between years, with the highest peak during the SarsCov2 pandemic. There was a positive correlation between MO and TM with a delay of about 9 weeks, indicating that heat stimulates the proliferation of mosquitoes. This result is consistent with the literature and may indicate heat island effects. A positive, but weaker, correlation emerged between MO and PL also after a 9-week delay. Thus, TM appears to be a factor predominantly associated with mosquito development, while PL appears to be more important for larval development. There was no significant correlation between MO and VS, which is corroborated by previous studies in Santos. There was no correlation between DH and MO. Previous studies also describe that *Aedes aegypti* has low oviposition in areas with higher HD that have already been urbanized, with HD being a more important factor in places of rapid and unorganized urbanization. There was a low negative correlation between NDVI and MO, indicating that vegetation cover has an antagonistic role, although weak, in the proliferation of mosquitoes. Taken together, these findings suggest when and where mosquito mitigation should be prioritized.

KEY-WORDS: Epidemiology. Culicidae. Dengue. Global Health. Urban Ecosystem.

ABBREVIATIONS LIST

AV	Green areas
DH	Human Density
FAA	Females per trap per area
IBGE	Instituto Brasileiro de Geografia e Estatística
IPVS	Índice Paulista de Vulnerabilidade Social
MOSQ	Mosquitoes
NDVI	Normalized Difference Vegetation Index
PopH	Human Population Density
PLUV	Pluviosity
SC	Census Sector
TEMP	Temperature
USGS	United States Geological Survey
VULN	Social Vulnerability

SUMMARY

1. Introduction

1.1. *Aedes aegypti*, a global health concern

The *Aedes aegypti* is one of the most studied mosquito species, given its medical importance and large spread throughout the world. Regardless of its uncertain origin, recent and well-established studies describe the possible ancestral of *Ae. aegypti* as natural from the Madagascar island. The first lineages of these mosquitoes might have spread from West Africa to the New World through commercial routes of the 15th century (Powell and Tabachnick, 2013; Soghigian, Gloria-Soria, Robert, et al*.,* 2020).

Since then, *Ae. aegypti* has become a pantropical species, predominantly urban and anthropophilic (Kraemer et al*.,* 2015). Its vectorial competence to several pathogens, notedly Dengue, Zika, and Chikungunya viruses lead it to be among the most important vectors. Additionally, the high genetic diversity of *Ae. aegypti* proportioned great adaptability over the centuries, contributing to leading this insect to become a global health concern (Suesdek, 2018). The World Health Organization (WHO, 2023) estimates that 3.9 billion people are at risk of infection only by Dengue virus, which represents approximately 50% of the global population.

Mitigation initiatives are on how to control and eliminate the *Ae. aegypti*. However, the high adaptability of this species to different environments turns this control into a complex challenge. Numerous studies suggest that environmental and social conditions may act as benefactors for mosquito dissemination, such as high temperatures and pluviosity, precarious urbanization, and low income. Despite that, many of those hypotheses are based on empirical evidence and just a few of them have tested the variables with statistical significance and entomological data. Then, it is crucial to understand the influence of environmental features on the population dynamics of *Ae. aegypti* in different cities for planning better sanitary control programs (Tun-Lin, Burkot, and Kay, 2000).

Another concern that leads the urgency for an assertive program to prevent and eliminate those infestations is the fact that there are no secure and efficient vaccines available for most of the viruses that the insect may transmit. In Brazil, there are approved vaccines only for Dengue and Yellow Fever, but the vaccinal coverture stills incomplete in the country for those viruses, considering the vaccination coverture established by the World Health Organization (Brazil, 2024a; Brazil, 2024b; Brazil, 2024c, WHO, 2024).

1.2. Temperature and pluviosity

Temperature is well known as one of the most influential factors for mosquito development. It is commonly associated with the development of the *Ae. aegypti* under their first life cycle stages, spread, and reproduction. In contaminated insects, temperature also leads to increasing virulence and a higher extrinsic virus cycle (Chouin-Carneiro and Santos, 2017; Couret, Dotson, and Benedict, 2014). Plenty of models forecast the growth of this species dispersion by the expansion of the tropics implied by climate change. However, it is still unclear how temperature would change mosquito populations in small models such as states and cities (Kraemer, et al., 2019; Naish, Dale, Mackenzie, et al., 2014).

Another concerning urban phenomenoun regarding *Ae. aegypti* infestation is the heat island effect. Heat islands are areas that are specifically warmer than their surroundings, especially in urban scenarios of high asphalt paving, low humidity, and seldom presence of green areas. It is uncertain if those regions could profit from the infestation or not. Shreds of evidence describe that oviposition in areas of extremely warm temperatures, usually above 35ºC, may be deleterious for this species (Tun-Lin, Burkot, and Kay, 2000). Otherwise, highly urbanized cities like Santos (Brazil) which usually harbor heat islands even during colder seasons, could lead mosquitoes to proliferate vigorously, especially in unfavorable climate conditions (Hii, Rocklöv, and Ng et al*.,* 2009; Porangaba, Teixeira, Amorim, et al., 2021; Heisler, Brazel, 2010; Bala, Prasad and Yadav, *et. al.,* 2019).

1.3. *Ae. aegypti* and human social conditions

Although the mosquito has a well-known preference for urbanized areas, how they are affected by the presence of human population is not well described. Human density frequently represent more blood meal sources and breeding sites, which are crucial for infestation maintenance. Social vulnerability is frequently linked as a factor for the occurrence of the mosquito. Still, there are too few studies to support this hypothesis and to explain how exactly social vulnerability could interfere with mosquito dynamics. The few researches that investigate any correlation (positive or negative), were performed in specific cities that do not comprise a representative amount of countries. From those few studies, the ones associating social vulnerability and human density with epidemiological data are also scarce. (Forattini, 2002, Bohm, et al*.,* 2023, Whiteman, et al., 2020, Silva *et. al*., 2017).

1.4. Soil coverage estimation by Normalized Difference Vegetation Index

More than 3.300 artificial satellites of diverse countries are currently in the Earth's orbit. Some of them are designed for specific functions such as military surveillance, Global Positioning System (GPS), weather estimation, forest fires vigilance, land use, and broadband. Each of those artificial satellites has specific resolutions, according to the function it was planned to have. Some of those characteristics define the spatial coverage of the scene, the number of days for revisiting a specific spot, the radiometric scale of the image, and the bands that are collected in each captured scene.

The NDVI is a mathematical estimation of vegetation cover according to the red and infrared reflectance in a determined study area. Satellites equipped with sensors for red and infrared waves capture Earth data that are analyzed using GIS (Geographical Information System) softwares. The final result is a number for each image pixel, varying from 1 to -1, where 1 is considered highly sensitive to infrared waves (presence of healthy vegetation) and -1 is highly sensitive to red waves (absence of healthy vegetation). In *Ae. aegypti* studies, NDVI is frequently used as a proxy to estimate breeding sites for mosquitoes and identification of local humidity and precipitation (Parselia et al., 2019; Kalluri, 2007; Estallo, 2016).

1.5. Investigation of *Ae. aegypti* in the study site Santos-SP.

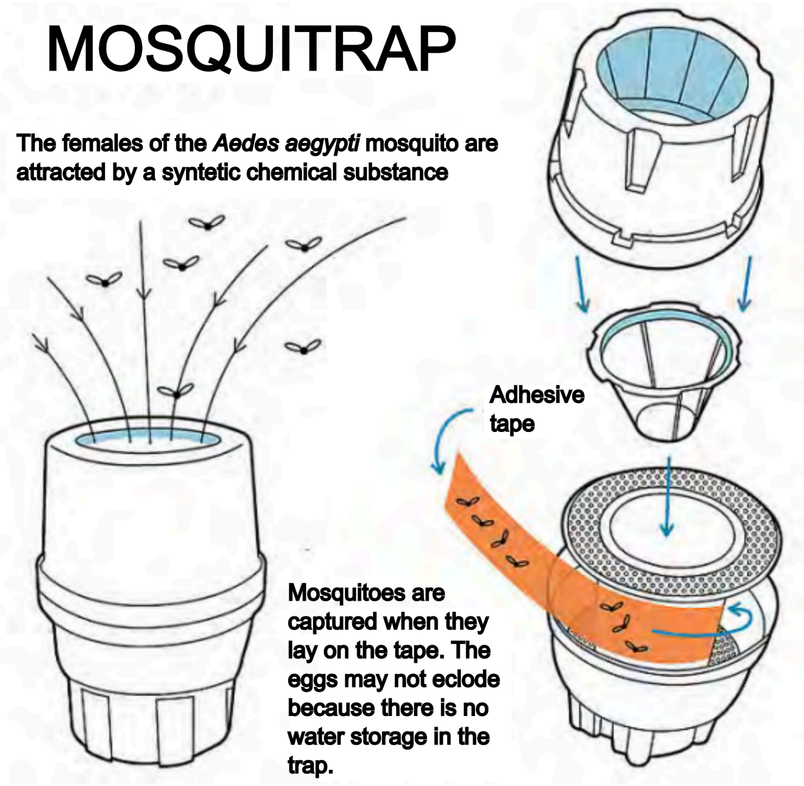
Santos is a city located in the central coast of the Sao Paulo State (SP - Brazil) that has the largest seaport in Latin America (Port of Santos, 2023). It is historically known as an “entry door” for exotic pathological agents (Barbosa, 2000; Lucio, 2017) and is estimated to be a gateway for new haplotypes for *Ae. aegypti* mosquitoes, turning the city into a repository for new viruses and new vectors.

Santos is also highly social and urban heterogeneous, which possibly contributes to the dissemination of arboviruses (Martins, 2014; Resendes, 2010).

In order to better understand how *Ae. aegypti* are spreading and to avoid epidemics, Santos´ City Hall instituted a surveillance program in the year of 2012 that is stills active. This program consists in *MosquiTRAPS* (Figure 1) distributed over the municipality that are weekly vistored. Data on how many female *Ae. aegypti* were collected are promptly sent to the local health surveillance and aid the decision-making on mitigation strategies (Santos, 2023b; Melo, Scherrer, and Eiras, 2012).

In the year 2021, the city had epidemics of Dengue and Chikungunya concurrently to the Sars-Cov-2 virus, and approximately 13,600 cases of arboviruses were reported in the city in that year´s second half. Most of those cases were infections of the Chikungunya virus, followed by Dengue fever and only one case of Zika. The beachfront zone of the city was the most affected (Santos, 2023).

Figure 1. ***Mosquitrap*** **scheme.**



Source: Adapted from Negreiros et al., 2011. There are actually 481 *MosquiTRAPS* spread throughout the city. Those traps were developed to attract gravid *Ae. aegypti* females to its interior for oviposition. The trap is round, with only one way for mosquito entry. It also has a strong adhesive tape attached to its laterals which prevents the females from flying back to the external environment. Once a week, Santos Health employees collect and identify the captured mosquitoes. They use a special mobile to send each individual trap analysis to an online system that updates city trap maps with different colors according to the number of females captured.

Figure 2. Santos city *Mosquitrap* distribution map as of the 39th epidemiological week of 2023.

Source: Santos' City Hall.

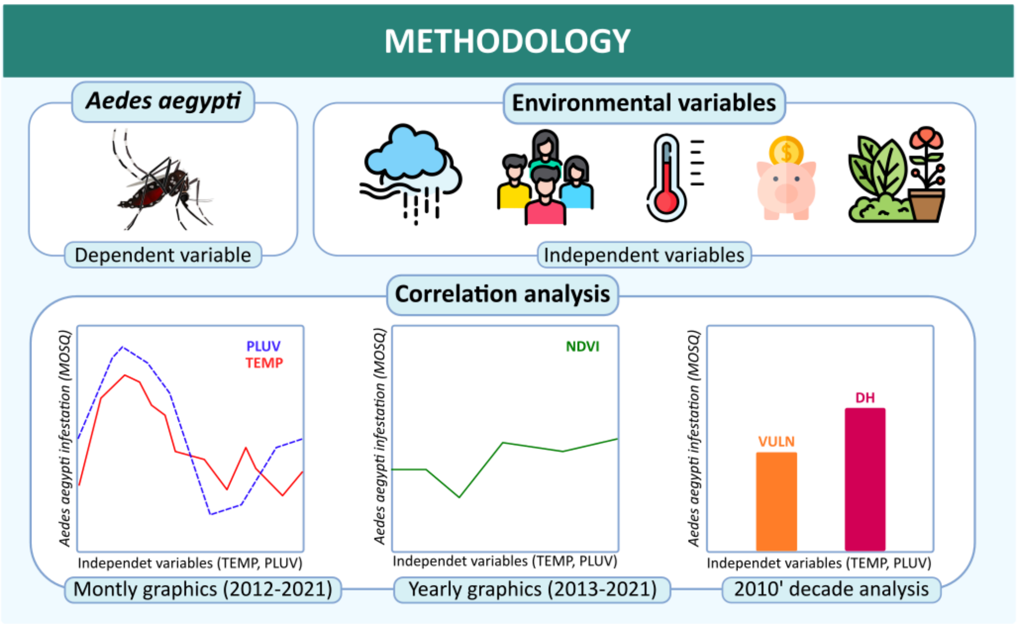
2. Objectives

The aim of this work was to investigate the correlation between *Ae. aegypti* infestation (dependent entomological variable) and Environmental independent variables (temperature, pluviosity, human density, human population, and soil coverage).

3. Methodology

The graphical abstract below summarizes the applied methodology, which will be better explained in the next topics, according to each variable.

Figure 3. Graphical abstract of the methodology. Results for correlation analysis are fictional and represented only for illustrative purposes.



Source: The author.

3.1. Trap Coverage for Census Sector

The trap coverage estimated the amostral representativity of each sector region aiming posterior standardization of data and the knowledge of the assertivity of trap distribution, once Santos' City Hall spread them. Each *MosquiTRAP* had its own coordinates, enabling the trap allocation in each census sector using the QGIS 3.26.1. Software.

A 150-meter buffer was delimited around the trap centroid, aiming to identify the areas where the most commonly considered flying range for *Ae. aegypti* was covered. The trap coverage for census sector was then defined by the total area of the census sector minus the area covered by the trap buffer.

3.2. Mosquito data acquisition and treatment

The original *Mosquitrap* data from 2012 to 2022 were provided by the Santos´ City Hall for this project after an agreement with Instituto Butantan, and Faculdade de Medicina da Univesity of Sao Paulo. The data were in spreadsheet formats with weekly information for each trap. Its columns had information about ID, address, geographic coordinates, year of inspection, and amount of collected *Ae. aegypti* females, state of inspection (accomplished or prevented), and inspection date. All those data were organized using the R software version 4.2.2.

A new spreadsheet was created with the columns “total female captured for coordinate”, “total different ID traps for coordinate”, and “year”. The data were also stratified in three temporal scales: epidemiological week, month, and year. This new database was updated posteriorly with “census sector identification” for each coordinate, “census region area”, “total human population for census region”, “social vulnerability for census region”, and “female density” (Densidade de Fêmeas, DF from now), and “and data on green and urban areas per census sector where the traps were located. The DF was defined considering the total number of females per trap per area of census region. This estimation was created in order to estimate the most accurate density of females per km² of the analyzed area.

3.3. Temperature data acquisition and treatment

Daily Celsius (ºC) temperature data from 2015 to 2021 was acquired with the Santos Civil Defense in spreadsheets. The whole historical series was gauged in the same geo coordinate point, in the extreme south of the city. Once the temperature was registered daily at 10-minute intervals, the original data was manipulated in the R software to generate a new daily spreadsheet where the temperature of each day was considered as the mean of the total observations for each day. Other two time scales were constructed. The first one was based on an epidemiological week scale. The second one was a monthly scale posteriorly compared with pluviosity data. There were no information about weekly or daily pluviosity.

3.4. Pluviosity data acquisition and treatment

Pluviosity data was first acquired with the Centro Nacional de Monitoramento e Alertas de Desastres Naturais (National Center for Natural Disaster Monitoring and Alerts - CEMADEN), that have 17 rain gauge stations distributed in Santos neighborhoods, and a historical range from 2014 to 2022. Nonetheless, all the data was discarded after the beginning of data cleaning, because the of lack of data in many following days, weeks, and sometimes months, which could strongly jeopardize the analysis. Then, monthly data were acquired by the Santos Civil Defense. Data had a historical range from 1940 to 2021, but only the range from 2016 to 2021 was used in the analysis in order to compare the influence of this variable with temperature and mosquito infestation.

3.5. Data acquirement of Social Vulnerability and Human Population

Social vulnerability index (VULN) was calculated based on data provided by the Fundação Sistema Estadual de Análise da Dados of São Paulo State (Seade - Sao Paulo State System of Data Analysis), that is the Índice Paulista de Vulnerabilidade Social (IPVS, Paulista Index of Social Vulnerability). The QGIS Software was used to allocate each VULN information to the georeferenced shapefile of Santos City. The R Software was used to aggregate data of VULN, census sector identification, and mosquitoes collected per analyzed area. Then this historical range from 2012 to 2021 was analyzed for their correlation. Santos City has a total of 671 census sectors (SC) but the absence of vulnerability or mosquito data induced data exclusion according to each correlation analysis, which is better described in the results and discussions.

The VULN is a complex index which provides an estimate of social vulnerability. It is composed of several social and demographic variables (Table 1). The VULN index was stratified in census sectors, which is the minimal geographic scale defined by IBGE. We used herein the VULN based on data from the 2010 Brazilian Census, developed by the Instituto Brasileiro de Geografia e Estatística (IBGE, Brazilian Institute of Geography and Statistics). The Census is usually updated at the begining of each decade, but could not be updated in 2020 due to political issues. Because of that, data used in this research are outdated and correspond to the decade of 2010. Either the VULN index and the isolated variables that comprise the index were tested for correlation with mosquito infestation (MOSQ).

Human population was estimated in this research following the Santos city population as defined by the IBGE.

Table 1. Variables that compose the IPVS index, 2010 version.

VARIABLES	
Socioeconomics	**Demographics**
Household income *per capita*	% of responsible people aged from 10 to 29
average income of the woman responsible for the household	% of responsible women aged from 10 to 29
% of households with per capita household income up to 1/2 MW	average age of responsible persons
% of households with per capita household income up to 1/4 MW	% of children aged from 0 to 5 years old
% of people responsible for the household who are literate	

Source: Adapted from SEADE. The full IPVS composition methodology is available at https://ipvs.seade.gov.br/view/index.php. Accessed in 12/14/2023, 15:51.

3.6. Analysis of Soil Coverage with NDVI (Normalized Difference Vegetation Index)

Landsat-8 satellite images were obtained from the United States Geological Survey (USGS) and treated with the QGIS Software. One image per year from 2015 to 2022 was selected for the NDVI composition so that its data were tested for correlation with MOSQ. The criteria for image selection were the disponibility in the USGS databank and the absence of clouds over the city. Another criterion was the equilibrium between a good scene and the most similar climate station. Thus, most images classified were from May. The satellite images of years 2015, 2016, 2017, and 2019 were taken in January, July, June and April, respectively. The mosquito data historical range began in 2012, but a failure in Landsat-7, the predecessor of Landsat-8, made it impossible to analyze the Santos region.

Five gradual categories of NDVI (1-5, being 1 water sources, 2-3 roof coverture and 4-5 vegetal cover) were defined in an attempt to differentiate water sources, building coverture (slab or tile), and vegetation cover (sparse or dense). The

NDVI images obtained were then converted from raster to shapefile, and the quantification of each pixel category per census sector was defined using the QGIS and R software. Lastly, we tested for any linear correlation between each category of vegetation cover and the respective female mosquitoes captures (MOSQ) index. All tests were done per census sector.

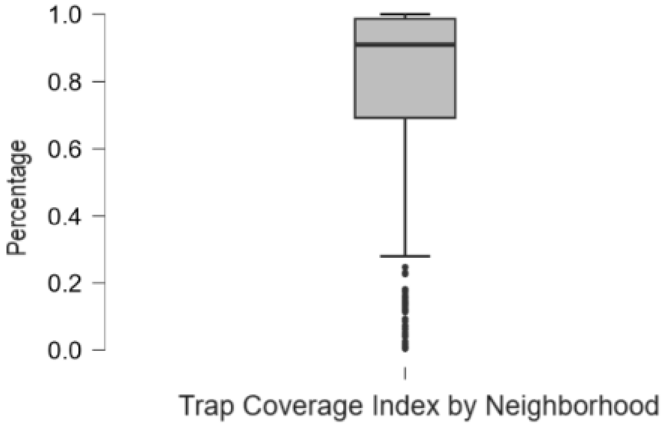
4. Results and Discussion

4.1. Trap Coverage Index by Neighborhood

It is known that *Ae. aegypti* mosquitoes have a limited flying range around their emerging site, which is generally restricted to 150 meters (McDonald (1977); Trpis & Hausermann (1986); Muir & Kay (1998). However, it is known that the mosquito-spread may reach 840 meters when there are no favorable breeding conditions near the emergin site (Reiter, Amador, and Anderson et al., 1995). Honório, Silva, and Leite et al. (2003) also suggested that females may be able to fly up to 800 meters, scattering the insect infestation.

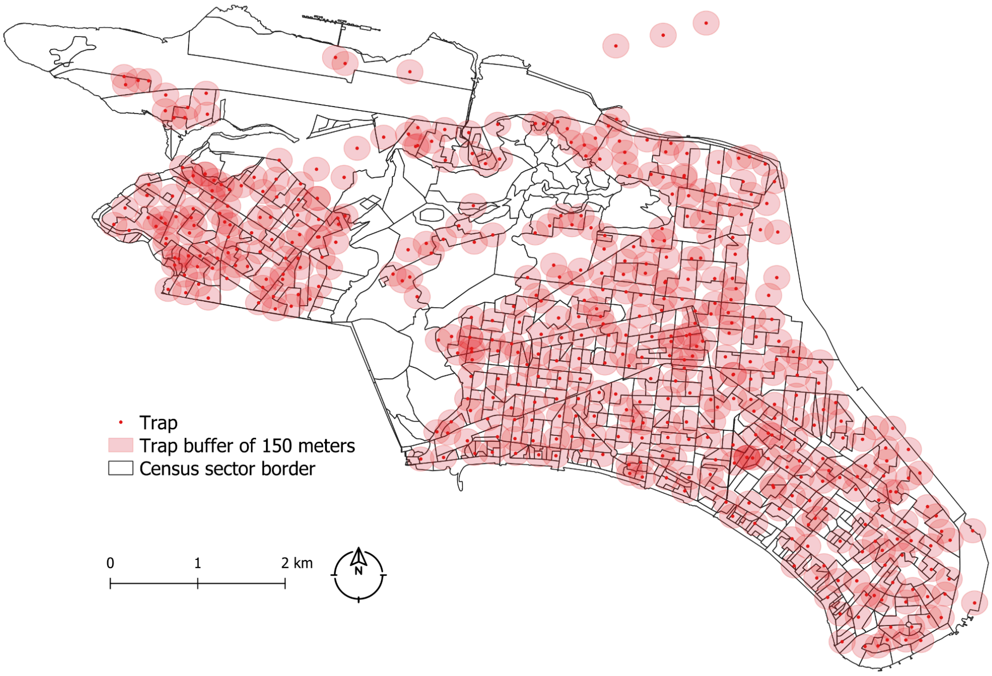
Considering the flying range discussed above, a buffer of 150 meters was delimited from each trap’s centroid. This conservative range was chosen considering the small area of the city and assuming the worst scenario for trap coverage. Figure 3 demonstrates that despite the discrepant amount of traps in each region, approximately 60% of the city area is actually covered by *MosquiTRAPS* (Figure 4).

Figure 4. Boxplot of the trap coverage (percentage of the area) for each census sector. Most of the census regions were determined to be around 90% covered by traps or their buffer. Few regions had 0.3 or lower coverage.



Source: The author.

Figure 5. Trap coverage in Santos City. Each trap was buffered with a 150-meter circle, estimating the limits of mosquitoes' flight. Darker areas are census sectors with more than one trap influence.



Source: The author.

This estimation was acquired considering the city area (37,791 km²) minus the sum of areas covered by the buffer disregarding the overlapped areas (22,744 km²). Places without coverage are in general regions of slums, dense vegetation, or seaports. The trap coverage and buffer coverage were calculated only for 2022. Once the accuracy of trap distribution was not in the scope of this research, this analysis was released as an endorser of the found results, and not as a critical review.

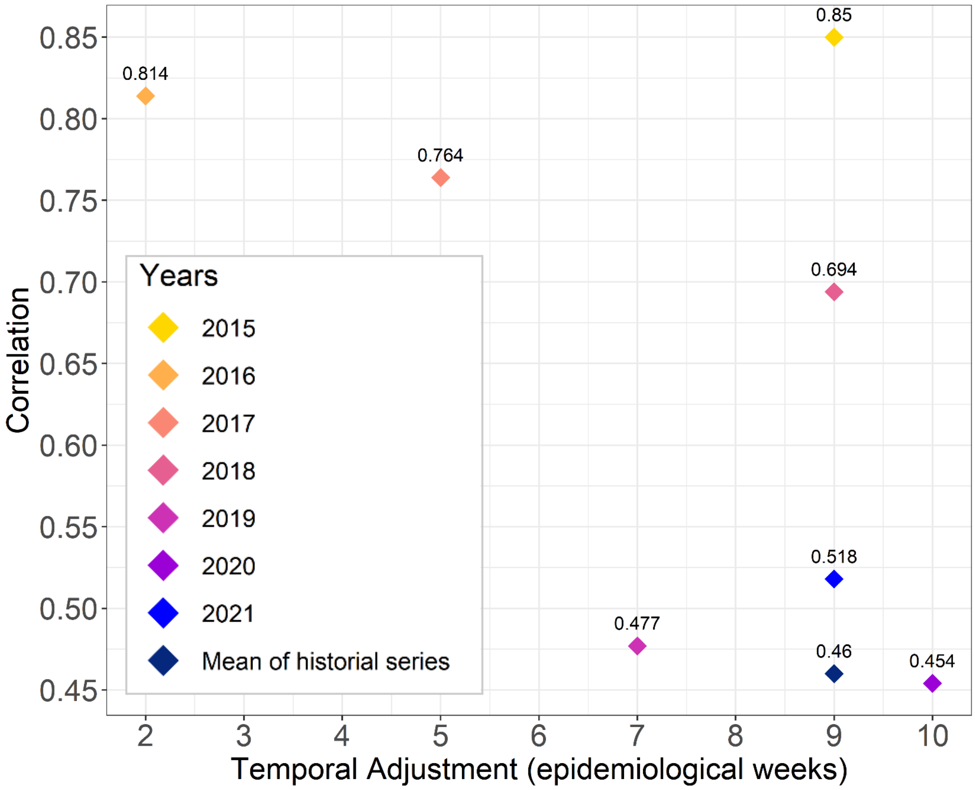
4.2. Temperature and Pluviosity

Aiming to better understand how temperature affects the mosquito´s population in Santos, a shift adjustment of captured females against daily temperatures was performed. The aim was to determine how many weeks after a temperature increase, a significant change in the mosquito population would occur.

According to the historical temperature series between 2016 and 2021, the mosquito demographics in Santos, represented by MOSQ took some weeks to mirror the temperature (TEMP) changes, in a direct proportion ($r = 0.46$, $p = 0.000$ ($F = 1.354$). Thus, a preliminary conclusion is that although temperature elevation raises mosquito proliferation, this phenomenon usually does not take place immediately.

Different years had different values of “weeks-shift” of the mosquito population. As shown in Figure 5, whereas a nine-weeks-shift was the best fit for the TEMP/MOSQ correlation, in 2016 the best fit was 2 weeks, indicating that 2016 had optimum temperature and other environmental elements for the insect to reproduce faster. From the observed data, one could say that the arithmetic mean shift time is 7.5 weeks.

Figure 6. Graphical presentation of shift intervals (in weeks) regarding the maximum correlation between TEMP and MOSQ.



Source: The author.

A similar result was obtained by Chaves et al. (2012), which registered an abrupt outbreak of *Ae. aegypti* in Thailand 10 weeks after an high-temperature event. In a slightly different situation, a mathematical model developed by Cheng, Bambrick, and Yakob et al. (2020), found a similar result when testing for the correlation between temperature and dengue outbreaks in China, concluding that heatwaves may delay dengue outbreaks, probably due to the decrease in mosquito population.

Another model created by Chaves, Scott, and Morrison et al. (2014) reinforces the negative effect of high temperatures on those *Culicidae* species and hypothesizes that they may have a very sensible and still not known evolutionary trade-off adaptation to dry seasons that triggers them to lay more eggs per container. Thus, this larval crowding could lead to low larvae survival and consequent population decrease, once overwhelmed containers are related to high larval mortality (Barbosa, Peters and Greenough, 1972). In the first moment, it would provoke a decrease in the mosquito population, followed by a build-up that could take some generations to reach the population equilibrium once again, generating the delayed outbreak.

This hypothesis is corroborated by Cheng, Bambrick, and Frentiu et al., (2021), which also underline a delayed dengue outbreak in almost 8 weeks after temperature increases and 12 weeks after extreme pluviosity events. Nevertheless, the existence of this trade-off evolutionary mechanism, or how temperature could exactly arouse it, is still uncertain (ABDALGADER et al., 2022).

Despite the importance of pluviosity, it is well established that the *Ae. aegypti* infestation is modulated by multifaceted factors, whereof we may never know exactly all of them, just like its separated influence in different environments (Reiter, 2001). Regarding correlation between PLUV and MOSQ in Santos, we found that the best fit was 2 months after the pluviosity event, as shown in Figure 6 (r = 0.28, F(1.80) = 7.08, p = 0.009392).

As well as temperature, pluviosity also acts as an important environmental variable for *Ae. aegypti* mosquitoes, essential for its first life stages: egg, larvae, and pupae. Aiming to understand how pluviosity affects this species in Santos, the same adjustment test run with temperature was run for pluviosity. As a limitation of the acquired data, it was not possible to time scale the correlation in epidemiological weeks, so it was scaled monthly.

Table 2. Graphical presentation of shift intervals (in weeks) regarding the maximum correlation between PL and MO.

Year	Variable	Shift	Correlation	p	t	df
2015	TM	-2	0.972	0.00269	11.649	8
	PL	-3	0.926	0.00034	6.468	7
2016	TM	-1	0.858	0.00072	5.016	6
	PL	-2	0.721	0.02834	2.754	7
2017	TM	-1	0.884	0.00031	5.658	9
	PL	-4	0.538	0.16890	1.564	6
2018	TM	-2	0.784	0.00725	3.574	8
	PL	-2	0.725	0.01768	2.977	8
2019	TM	-2	0.642	0.04528	2.37	8
	PL	-2	0.482	0.15780	1.558	8
2020	TM	-4	0.689	0.05891	2.327	6

	PL	-4	0.722	0.04316	2.556	6
2021	TM	-2	0.572	0.08421	1.971	8
	PL	-2	0.628	0.05201	2.281	8

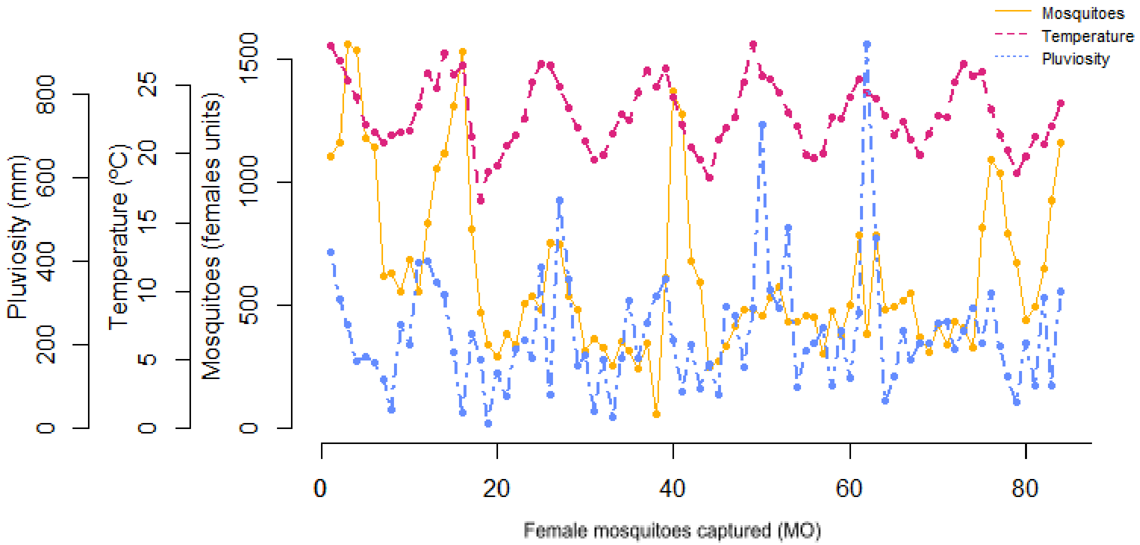
Source: The author.

Kakarla, Caminade, and Mutheneni et al. (2019) described that the highest correlation they found between pluviosity and Dengue cases had r = 0.32, modulated by a non-linear relationship of 12 weeks, and was also highly influenced by temperature. Despite the fact that an increase in pluviosity leads to an increase in breeding sites, events of extreme pluviosity may overflow mosquito containers, leading to a population reduction (Morin, Comrie, and Ernst, 2013; Chien and Yu, 2014).

The same delay of 8 weeks of influence for both temperature and pluviosity was described before but for the occurrence of Dengue Fever and Dengue Hemorrhagic Fever (Yu, Guo and Lung, 2007; Arcari, Tapper and Pfueller, 2007), which may imply in mosquito population increase.

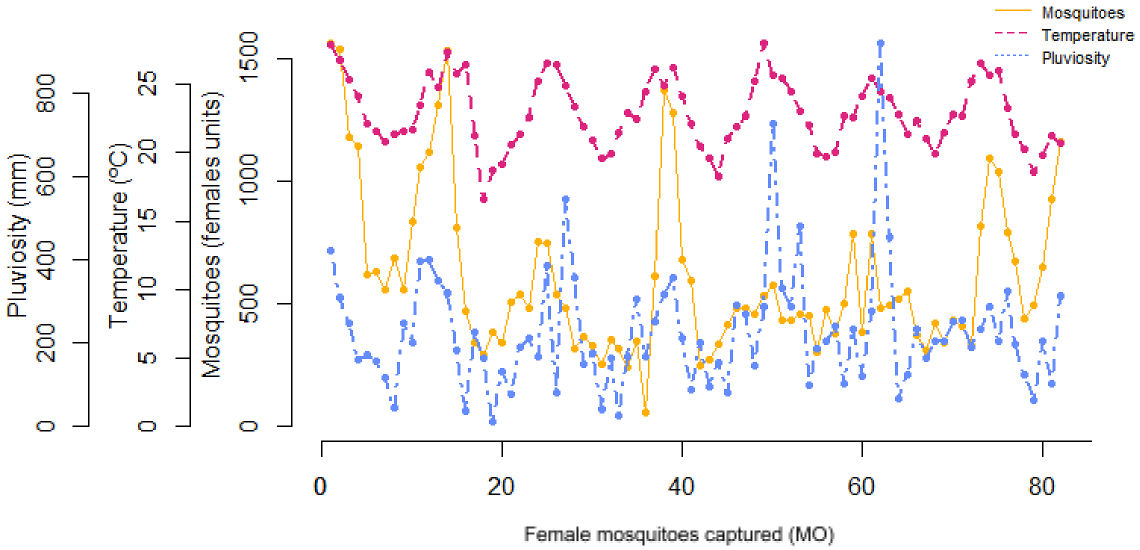
Thus, while pluviosity seems to be linked to breeding sites and the initial development of the insect, the temperature seems to act as a more important factor. Some authors suggest the temperature increase as an influencing factor for a possible evolutionary trade-off for regulating populational survival on *Ae. aegypti*. However, such a mechanism was not comproved until the finish of this work. Considering that Santos is a small city, highly urbanized seaport city and potential receiver of new genetic variants of *Ae. aegypti* throughout commercial ships, it becomes necessary to implement strong and well-established surveillance policies to mitigate this insect over the years in order to better understand its dynamics and better control the incidence of disease cases.

Figure 7. Historical standardized values for temperature, pluviosity, and mosquitoes registered from 2015 to 2021 without “shift weeks” adjustment.



Source: The author.

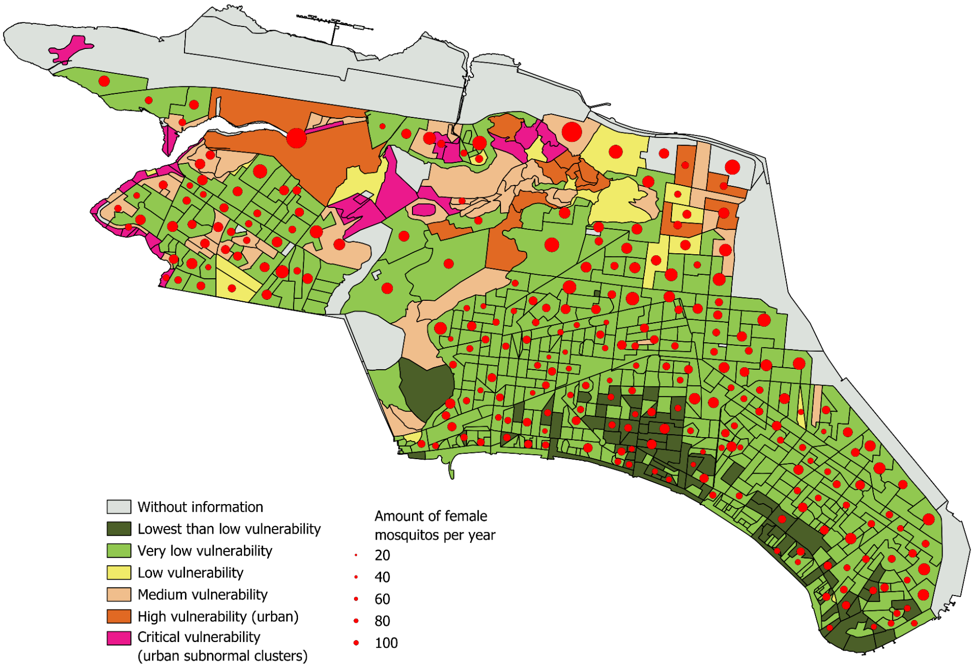
Figure 8. Historical standardized values for temperature, pluviosity, and mosquitoes registered from 2015 to 2021 with “shift weeks” adjustment.



4.3. Social vulnerability and mosquitoes (VULN x MO)

Santos has a total of 671 sensus sectors (CS) distributed inside its 37 km² of insular portion, but only 633 were considered for this analysis. 38 CS were excluded because of the absence of VULN or MOSQ data for those regions. All considered CS are represented in figure 8.

Figure 9. Map of Vulnerability in Santos in 2010. According to each census sector and visual sum of females, *Aedes aegypti* were captured between 2012 and 2022.



Source: The author.

Before the correlation test, the Shapiro-Wilk analysis was run to investigate the normality of data. It was possible to conclude that none of the vulnerability levels had a normal distribution. The skewness formed by the data distribution was also high. Because of that, the nonparametric Spearman correlation was chosen for this hypothesis test.

The results obtained after a Spearman correlation test revealed a very low correlation with $r = -0.06$ and $p\text{-value} < 0.001$. We then conclude that social vulnerability, although significant, is not a relevant variable in the infestation of *Aedes aegypti* mosquitoes in Santos.

Despite its small size, Santos is a very urban and socially heterogeneous city, with a very discrepant number of census sectors at each level of vulnerability. It is possible that those discrepancies had an influence on this result, once the number of CS for each level of vulnerability was very dissimilar**.** The descriptive statistics obtained by this unbalanced data are shown in figure 9 and highlight the difference in representation in the social vulnerability levels.

In another study by Santos and Ribeiro (2021) also in Santos, the IPVS and other social indicators were tested for their correlation with confirmed cases of dengue fever, mosquitoes captured per trap per area, and larvae captured. Only a few indicators had a significant result but in specific years, suggesting no important correlation.

Table 3. Descriptive statistics for females per vulnerability group.

	vulnerability groups					
	1	**2**	**3**	**4**	**5**	**6**
Mean	8.4	12.7	14.5	14	18.1	1.9
Std. Deviation	13.63	21.07	22.14	31.6	43.55	7.14
Skewsness	2.42	3.13	2.22	4.74	4.12	5
Shapiro-Wilk	0.674	0.647	0.705	0.487	0.467	0.301
P-value	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001
Minimum	0	0	0	0	0	0
Maximum	98	240	138	309	335	64

Source: The author.

In contrast to this nearly null correlation, Azevedo, Bourke, and Piovezan et al*.,* 2018 found a relation between larval *Aedes aegypti* and places with low social vulnerability, especially those places associated with high vulnerability and the presence of heat islands. Hagenlocher, Delmelle, and Casas et al*.* (2013) also had interesting results of positive and robust correlations while comparing Dengue disease cases and social vulnerability.

It is uncertain if the vulnerability classification as it was calculated by IPVS is the most adequate to infer connection with *Ae. aegypti* mosquitoes, especially because the index was not developed for such use. No significant set of results was found by Santos and Ribeiro (2021) using this index. Lopes, Silva, and Pelarigo et al. (2022) searched for a spatial correlation between IPVS by CS and dengue cases, constituting a dengue risk map with significant results, but instead of correlation, they searched for autocorrelation with Moran I.

Some researchers have had success using a Water-Associated Disease Index as an approach for identifying areas susceptible to the Dengue virus (Dickin, Schuster-Wallace, and Elliott, 2013; Pam, Nguyen, and Vu et al*.,* 2018; Dickin and Schuster-Wallace, 2014).

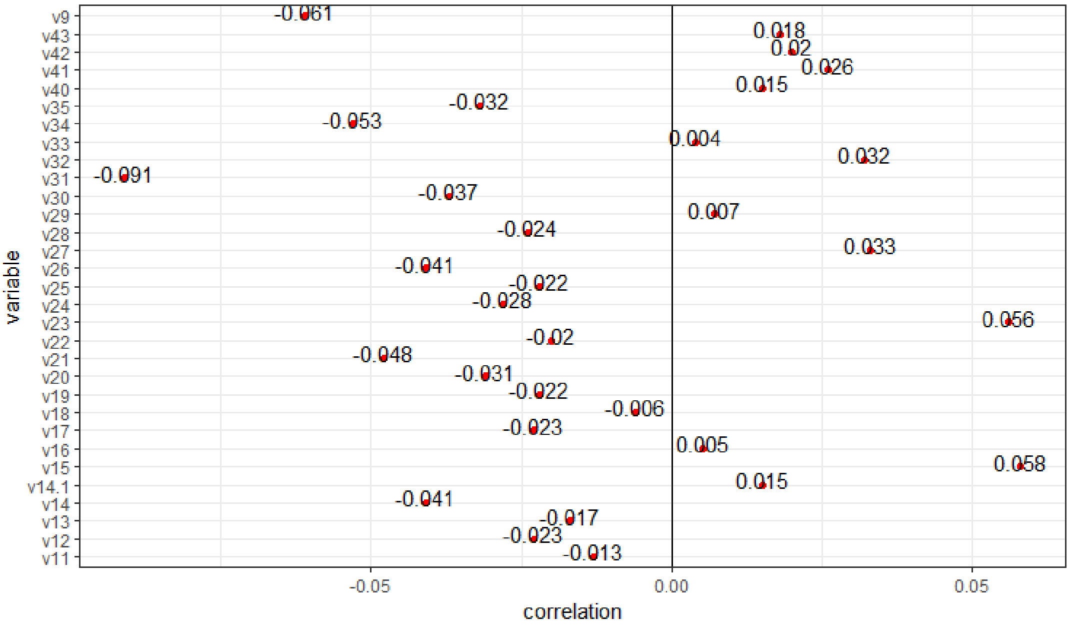
Another consideration that should be taken is that like temperature and pluviosity, most of the studies consider the number of disease cases instead of insect prevalence. Those who consider entomological factors are usually larvae caption data, which could be less assertive (Melo, Scherrer, and Eiras, 2012).

Vikram, Nagpal, and Pande et al*.* (2015) are one of the studies that use captured mosquitoes. They could conclude that dengue disease was inversely related to the income ratio of each locality. Despite that, it wasn't discussed if regions with low-income ratios also had a significantly higher number of mosquitoes captured.

This association between poverty areas and mosquito prevalence is also explained by Souza, Nazare, and Argibay et al*.* (2023), in a study where a low-income community was investigated for the presence of insects and places that could act as breeding sites. They found a high heterogeneity of places where the insect could reproduce, especially in public areas. They also mark how other low-income places usually have the same sanitary and social conditions, which is necessary to reinforce the vigilance in these places.

Some variables used to compose the social vulnerability index as described by IPVS, were independently tested for correlation with the FAA (Female per Trap per Area) index for each SC. Those variables was collected by the IBGE. Figure 9 describes the correlation between each of those social-demographic variables and the FAA.

Figure 10. Correlation between FAA and socio-demographic variables.



Source: The author.

Table 3. Socio-demographic variables tested individually as described by IPVS.

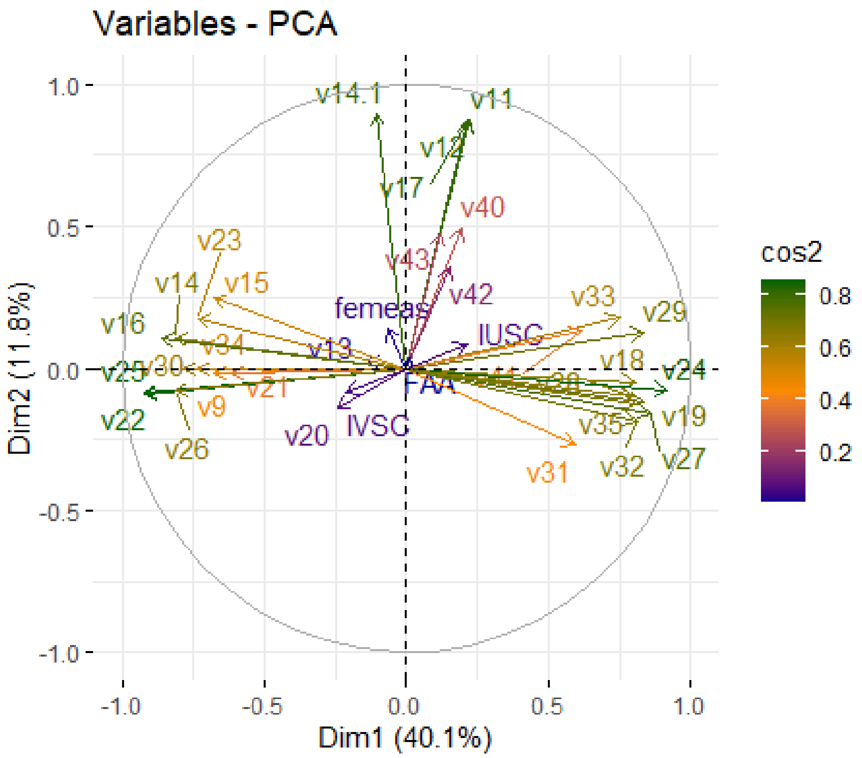
Code	Description
v9	Is the sector a subnormal cluster?
v10	IPVS Group
v11	Private and collective households
v12	Permanent private households
v13	Total number of improvised private homes
v14	Residents in permanent private homes
v15	Average number of residents in permanent private homes
v16	Proportion of children aged 0 to 5 years in the population
v17	Total people responsible
v18	Average household income of permanent private households
v19	Per capita income in permanent private households in the census tract
v20	Proportion of Households without Income per Capita
v21	Proportion of Households with Per Capita Income up to 1/8 SM - Proxy up to 70
v22	Proportion of Households with Per Capita Income from 1/8 to 1/2 SM - Proxy from 70 to 261
v23	Proportion of Households with Per Capita Income from 1/2 to 2 SM - Proxy from 261 to 914
v24	Proportion of Households with Per Capita Income of more than 2 SM - Proxy of more than 914
v25	Proportion of private households with nominal monthly income of up to 1/2 S.M.
v26	Proportion of private households with nominal monthly income of up to 1/4 S.M.
v27	Average age of responsible persons

v28	Average income of the head of the household
v29	Proportion of literate responsible people
v30	Proportion of responsible people aged 10 to 29
v31	Share of income of the head of the household in household income (in percentage)
v32	Average age of women responsible for the household
v33	Proportion of literate women responsible for the household
v34	Proportion of women responsible for the household under 30 years old
v35	Average income of women responsible for the household
v36	Factor 1
v37	Factor 2
v38	Rural Factor 1
v39	Rural Factor 2
v40	Proportion of permanent private households with water supply from the general network
v41	Proportion of permanent private households with a bathroom for the exclusive use of residents or a toilet and sewage system via the general sewage or rainwater system or via a septic tank
v42	Proportion of permanent private households with garbage collected by cleaning service or cleaning service dumpster
v43	Proportion of permanent private households with electricity
v44	Population living in collective housing - Municipality
v45	Population living in improvised private homes - Municipality
v46	Population residing in permanent private homes - Municipality
v47	Population residing in rural areas - Municipality
v48	Population residing in urban areas - Municipality
v49	Total resident population - Municipality

Source: The author. The variables that had a negative correlation with FAA as illustrated in figure 9, are highlighted in red.

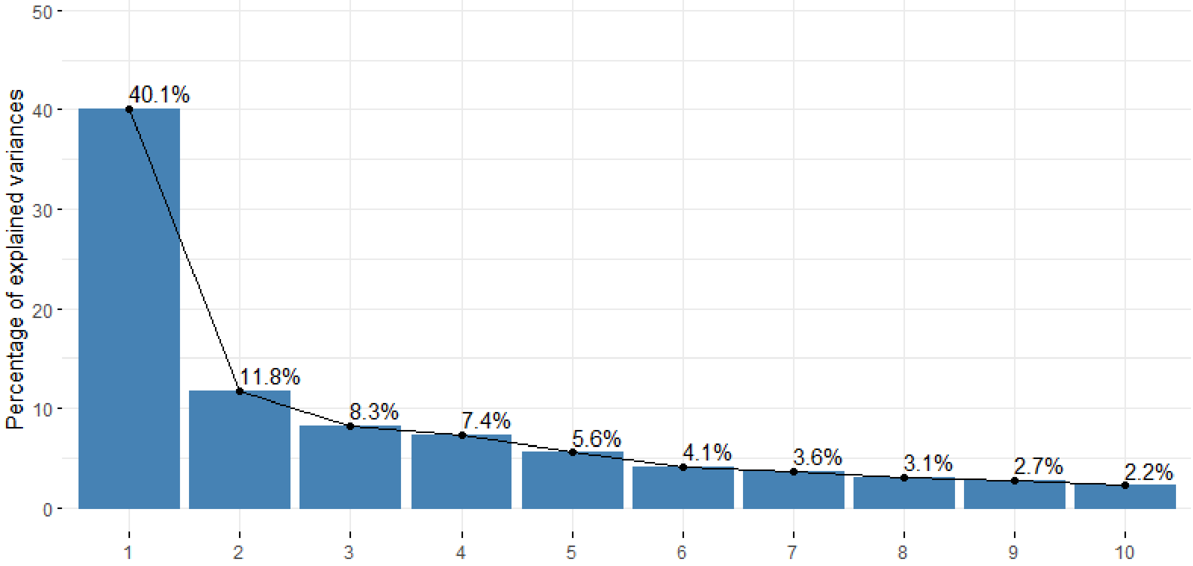
The Principal Component Analysis revealed that variables v11 (private and collective households), v12 (permanent private households), v17 (total people responsible), v14.1 (residents in permanent private houses), v40 (Proportion of permanent private households with water supply from the general network), v42 (Proportion of permanent private households with garbage collected by cleaning service or cleaning service dumpster) and v43 (Proportion of permanent private households with electricity) had some covariance with *Ae. aegypti* indicators (females, FAA), suggesting a possíble relation amongst them. Moreover, the first 5 PCA described 73% of the data variance, as represented in figure 11 and table 4. Those variables are related to lower social vulnerability conditions and could indicate that the worst *Ae. aegypti* infestation in Santos occurs in the better socioeconomical census sectors. However, the correlation was too weak to support this hypothesis.

Figure 11. Diagram of Principal Component Analysis. Colors represent the contribution of each variable for the PCA 1 and 2 (Dim1 and Dim2, respectively) after the Cos² calculation.



Source: The author.

Figure 12. The most significant CPA.



Source: The author.

Table 4. Values found after the CPA analysis.

CPA	Eigenvalue	Variance (%)	Cumulative Variance (%)
1.00	14.03	40.10	40.10
2.00	4.13	11.79	51.89
3.00	2.89	8.25	60.14
4.00	2.58	7.36	67.51
5.00	1.95	5.58	73.08
6.00	1.44	4.10	77.19
7.00	1.26	3.61	80.79
8.00	1.08	3.08	83.87
9.00	0.95	2.71	86.58
10.00	0.78	2.23	88.80
11.00	0.61	1.75	90.55
12.00	0.55	1.57	92.12
13.00	0.45	1.29	93.42
14.00	0.38	1.08	94.50
15.00	0.29	0.83	95.33
16.00	0.27	0.78	96.10
17.00	0.24	0.69	96.79
18.00	0.23	0.66	97.45
19.00	0.20	0.58	98.03
20.00	0.16	0.46	98.49
21.00	0.13	0.37	98.86
22.00	0.10	0.28	99.14
23.00	0.07	0.20	99.34
24.00	0.06	0.18	99.51
25.00	0.06	0.16	99.68
26.00	0.04	0.12	99.80
27.00	0.03	0.09	99.89
28.00	0.02	0.05	99.94
29.00	0.01	0.03	99.97
30.00	0.01	0.02	99.99
31.00	0.00	0.01	100.00
32.00	0.00	0.00	100.00
33.00	0.00	0.00	100.00
34.00	0.00	0.00	100.00
35.00	0.00	0.00	100.00

Source: the author.

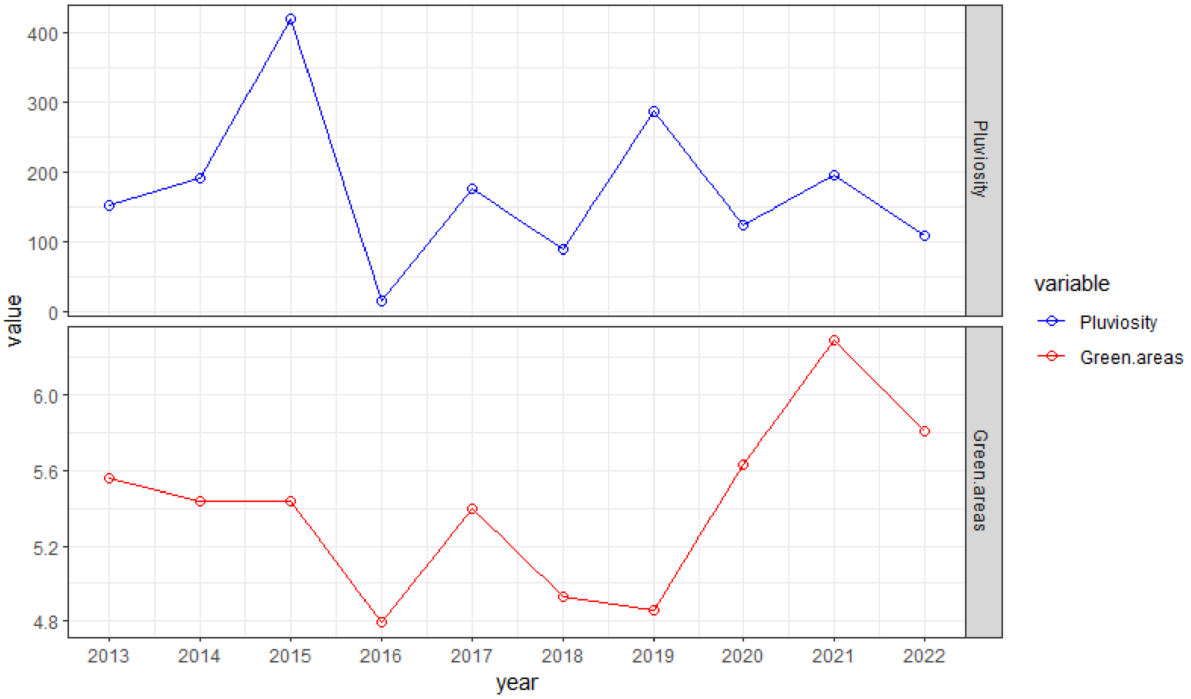
4.4. Normalized Difference Vegetation Index and Mosquitoes (NDVI x MOSQ)

The Landsat 8 spatial resolution was demonstrated to be inefficient for differentiating roof coverage in Santos buildings through NDVI methodology. Therefore, categories 2 and 3 (previously aimed to differentiate roof cover) were joined into the “urban” category while categories 4 and 5 (previously aimed to differentiate vegetation density) were considered simply as Green Areas (AV - Áreas Verdes). Category 1 was excluded from analysis, once it represented mostly the sea and very seldom detected water sources e.g. a lagoon.

The spatial resolution of this satellite was also inefficient for detecting the NDVI of some small census sectors. Because of that, some of them had to be excluded from the correlation analysis. Thus, from the original 671 SC, only 629 for each year from 2013 to 2022 were used for the analysis. There is a possibility that the 30-meter spatial resolution of the Landsat 8 satellite used is probably not the most effective for detecting sparse spots of green areas in a small and very urbanized city such as Santos. Figure 10 exemplifies the NDVI for the year 2020, with the Mosqui*TRAPS* positioned according to its geolocalization.

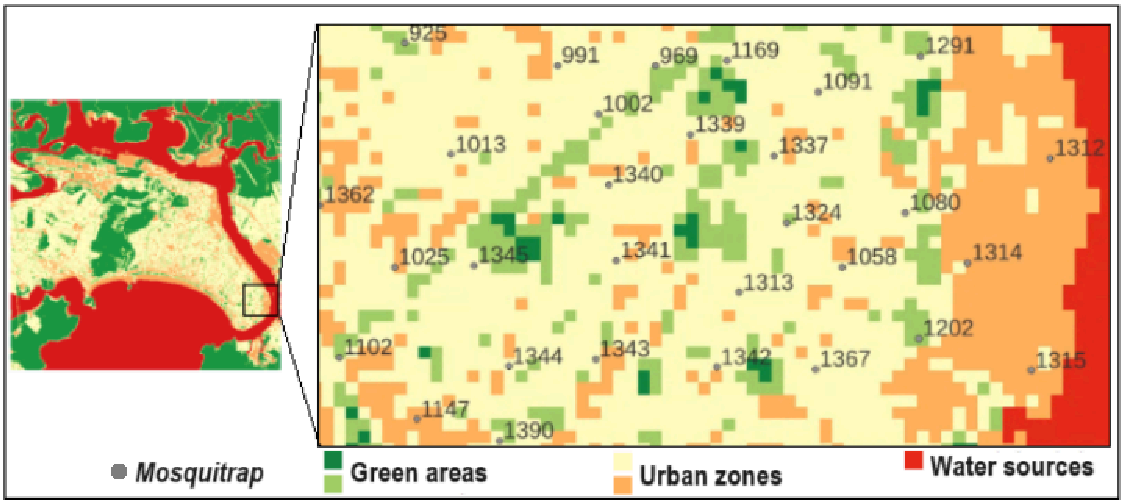
Satellite images were collected usually in May, but some years there were no available scenes for this month. In order to better clarify the origin of the images, figure 12 describes the month of each of them used in this research. Once previously pluviosity could also affect the NDVI results, the same figure 12 illustrates the difference in mean pluviosity for each scene captured.

Figure 13. Mean pluviosity in millimeters (mm) and NDVI for each studied year.



Source: The author. a) Precipitation in mm for each year studied. b) NDVI registered for the month that it was possible to have a satellite image.

Figure 14. Mapping of 5 urban classes in Santos, according to NDVI (February 2020), and positioning of traps. On the left is the entire island portion of Santos, and on the right is a highlighted rectangle with one fragment of the area analyzed in this work. Each image pixel was colored according to the NDVI results and indicates the presence of green areas, urban areas and water sources. Numbers are ID's for *MosquiTRAPS* positioned.



Source: The author.

Every census sector had its percentage of green area calculated considering the total number of green pixels inside its delimitation and the total area of the same analyzed region. It was possible to define that in the whole historical series each SC had around 5% green areas (std deviation = 0.166). The mean density of female mosquitoes was 233 per SC (acquired by DF calculation, std deviation = 542.335). The highest percentage of DF occurred between the Sars-Cov-2 pandemic years of 2021 and 2022. Together, those years had an increase of 120% considering the mean historical density of 233 females per SC (figure 11).

Table 5. Mean of green areas (AV) and female density (DF) per SC. DF % is the percentage of increase or decrease (highlighted in red) of female density, according to the historical mean density of 233 females per SC.

Year	AV	FAA	FAA (%)
2013	0,05	177,9	-23,67
2014	0,05	238,08	2,15
2015	0,05	280,44	20,32
2016	0,07	229,01	-1,75
2017	0,06	145,44	-37,6
2018	0,06	181,58	-22,09
2019	0,06	153,49	-34,15
2020	0,05	179,07	-23,17
2021	0,04	253,03	8,56
2022	0,05	492,75	111,41

Source: The author. Years with less captured females for the expected historical mean are highlighted in red.

The correlation of AV and DF in the historical series was $r^2 = -0.01$ (Spearman, p-value = < 0.001). Thus, it was possible to conclude that the presence of green areas had a -1% negative correlation with the mosquito population in each SC. In an individual analysis, none of the years were positively correlated with AV or had a r^2 value higher than 0,01.

This negative correlation differs from what has been reported in the literature recently. In a study carried out in Argentina, it was described that *Ae. aegypti* is able to lay eggs and develop inside tree-flooded holes in urban areas (Mangudo, Aparicio, and Gleiser, 2015). There are no studies like that in Santos City, but the nearly null correlation found between IVSC and DF could be an indicator that this phenomenon is not happening there.

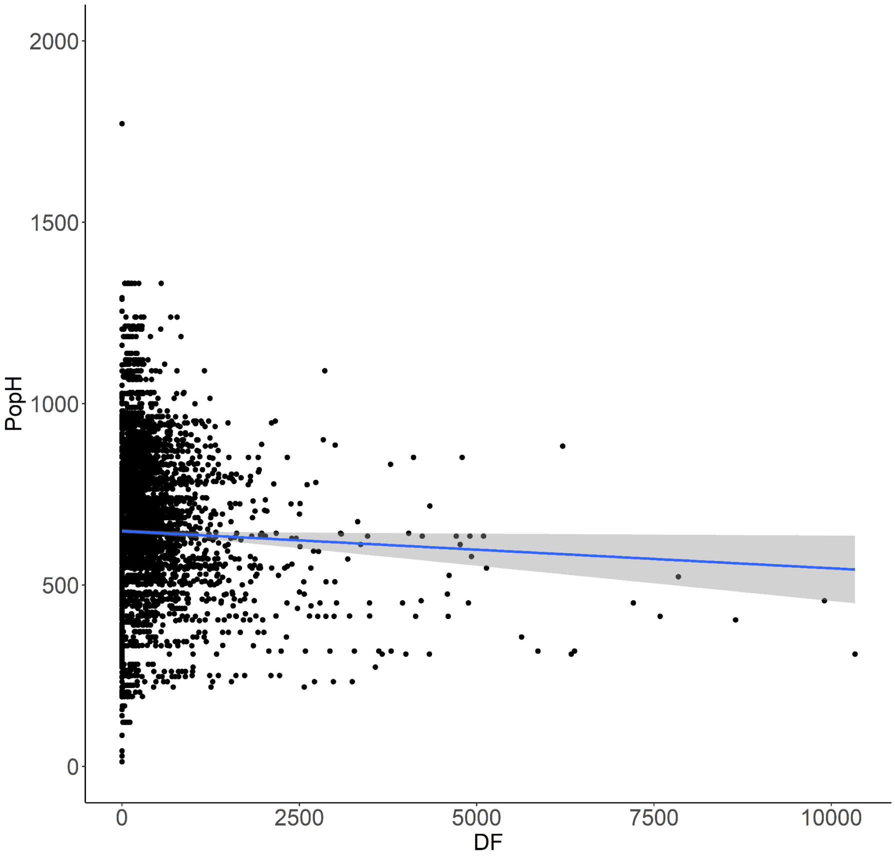
Another study describes how *Ae. aegypti* may be found in transition regions between urban and forest areas (Honório et al., 2009). As related previously, almost 60% of Santos is covered by green areas, which are mostly concentrated and occupied by some sparse vegetation and areas of natural forest. All of those regions are very close to the urbanized areas and could also be spots for *Aedes aegypti* reproduction.

Similarly, it was found that urban parks of São Paulo (São Paulo - SP) with loss of mosquito species due to the loss of their habitats may lead to the repopulation of those green urban areas with ecologically similar mosquito vectors living near those places. This relation seems to be stronger for *Ae. aegypti* when associated with small parks (Medeiros-Sousa et al*.* (2017). According to the Santos Municipality (Santos, 2023), the city has 6 urban parks, despite other green areas such as squares and natural forested places, but its mosquito population is not described.

4.5. Human demography x mosquito demography.

Correlation analysis was run with DF data from 2012 to 2022. Out of the 671 SC, 30 were excluded for lack of DF or Human Population Density (PopH) information. The correlation test resulted in r = -0.026 (Pearson, r^2 = 0,000676, p-value = 0.029), indicating a negative and significative relationship, where the increase of the human population leads to decay in the mosquito population. Despite its significant value, the correlation is too small and can indicate that this relationship is not the most important for *Ae. aegypti* infestation in Santos city.

Figure 15 - Correlation between Female Density and Human Population.



Source: The author. PopH: Population density. DF: Female density. The line represents the tendency of the negative correlation, while the shadow is the 95% confidence interval.

A similar negative relation was already noticed in Buenos Aires (Argentina) when it was found that *Ae. aegypti* mosquitos have a lower oviposition at higher human density areas (Carbajo, Curto, and Schweigmann, 2006). Despite that, the most frequently registered is the positive correlation between the human population and mosquito infestation, especially in studies considering domestic mosquito observations (Rodrigues et al., 2015).

The human population effect seems to be linked to the initial spread of the insects and the posterior spread of viruses caused by people's mobility through the transition from rural to urban areas. As suggested by Man et al. (2023), new cities development and the improvement of cities already established seem to be more important than the study of human density.

In agreement, rapid and not organized urban spread that constitutes poor communities of low income and high human population are also frequently linked to the *Ae. aegypti* spread. The spread and notification of diseases transmitted by *Ae.*

aegypti was also registered as correlated with human density, which could also suggest new studies in that field in Santos (Kolimenakis et al*.,* 2021).

5. CONCLUSIONS

- The *MosquiTRAPS* are properly distributed at the continental area of Santos city, providing a representative monitoring system on *Aedes aegypti* mosquitoes infestation.
- Temperature change seems to have a delayed effect of ~7.5 weeks on *Ae. aegypti* population in Santos city, possibly driven by mosquito development time.
- Pluviosity change seems to have a delayed effect of 9 weeks on *Ae. aegypti* population in Santos city, but the reasons for this are unclear and should be investigated in further studies.
- The Social Vulnerability seems not to be an impacting factor of *Ae. aegypti* infestation in the city, highlighting the need for people from all social levels to be committed with mosquito control..
- The Soil Coverage Analysis with NDVI indicated a low but negative correlation between absence of green areas and increase in *Ae. aegypti* infestation.
- The Human Demography has a marginal importance to the *Ae. aegypti* infestation in the city.
- New researches concerning those and others variables shouldbe accomplished in Santos in order to understand and explain the *Ae. aegypti* infestation dynamics on this coastal area.

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[1] According to Vancouver System.

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