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Identification of locations at risk for introduction of Asian Tiger Mosquitos (*Aedes albopictus*) with ground traffic transport in the Netherlands

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Foreword

Foremost, I would like to express my gratitude to my supervisors, Aldo Bergsma, and Adolfo Ibáñez-Justicia, for their valuable support and guidance throughout my thesis. Their feedback on draft texts and research ideas proved to be immensely helpful in shaping my research and refining my ideas. Additionally, I am grateful to Lotte van de Langerijt for kindly sharing her thesis data, which served as a crucial starting point for my own research.

Furthermore, I would also like to extend my gratitude to my fellow students who assisted me with writing, staying focused, and providing feedback on my texts and midterm presentation. Additionally, I am thankful for my friends and family who encouraged and supported me, and always showing interest in my progress during the thesis period, which helped me to stay focussed and motivated.

Jorrit Millenaar

Reader's guide

The research consists of one main research question and three sub-research questions, derived from the overarching main research question. While these sub-questions can be viewed individually, they collectively contribute to addressing the overarching research question. To facilitate a better understanding, some directions are provided to guide the reader more efficiently through the structure of this report.

After the abstract, the table of contents, and the introduction, where the research subject is introduced and the problem is addressed along with the main research question and sub-research questions, the methodology section will be outlined. The methodology will be presented for each sub-research question individually, while also considering common links between them. Initially, an overview of the overall research methodology will be provided, including a description of the methodology, data sources, timeframe, and study area. The study area description will delineate the various research areas related to each sub-research question, ranging from a broader scale in the first sub-research question to considerably smaller scales up to the third sub-research question. This underscores the distinctiveness across the sub-research questions. Subsequently, detailed methodology descriptions will be provided for each sub-research question.

The results will be presented similarly, providing an overview of the findings for each sub-research question individually. While there may be common links among the results of the sub-research questions, the primary emphasis will be on presenting them separately. In the discussion, recommendations, and conclusion sections, each sub-research question will also be addressed separately, with a final paragraph dedicated to the overarching recommendations and conclusions for the entire research. The conclusions encompass insights from all sub-research questions, ultimately contributing to a comprehensive answer to the main research question.

Abstract

The Asian Tiger Mosquito (*Aedes albopictus*) has rapidly spread across the globe over the past few decades. Its spread in Europe, facilitated by passive dispersal pathways like ground vehicles, could pose significant public health risks due to its capacity as a vector for various arboviral diseases. However, there is a lack of localized studies providing specific evidence to identify the extent to which ground vehicle transportation contributes to the introduction and spread in new areas. The aim of this research was therefore to identify potential risk zones for the introduction and distribution of *Aedes albopictus* in the Netherlands via this pathway.

The main datasets utilized in this study include distribution data of *Aedes albopictus* in Europe at the NUTS level 3 scale, along with postcode-based mosquito findings data for the Netherlands. These were combined with datasets of highways, holiday road traffic flows and population density. The study evaluated the range expansion of the mosquito by computing average distances of its movement, utilizing solely established regions, and regions determined as *Aedes albopictus* hotspot locations as identified by a Local Moran's I analysis. Two methods were employed to calculate the average distances of the mosquito's movement. Furthermore, the study examined the relationships between mosquito establishment categories (established, introduced, and absent regions) and holiday road traffic flow destined for the Netherlands, specifically focusing on highways. Lastly, high-risk areas in the Netherlands were identified by combining prior results, along with the postcode-based findings of *Aedes albopictus* in the Netherlands and population density.

The results revealed a range expansion of *Aedes albopictus* in Europe with average annual distances ranging from 75.88 km to 84.15 km and 335.46 km to 353.76 km, corresponding to the two methods used. Highway densities were observed to be the highest for European regions where the mosquito is classified as introduced, especially in recent years. Introduced regions like in Belgium, showed a strong correlation (0.82) between highway density and holiday road traffic flow to the Netherlands. Despite receiving only 6% of total holiday road traffic, in the latest years the Netherlands receives increasing holiday road traffic from introduced regions. The provinces of Zuid-Holland and Noord-Holland obtain the highest holiday road traffic overall and from introduced – and established selected European regions. Strong correlations between incoming holiday road traffic flow and population density in the Netherlands are observed for each establishment category: established (0.72), introduced (0.69), and absent (0.67). Furthermore, predictions suggest potential *Aedes albopictus* establishment in the whole of the Netherlands by 2028.

In conclusion, this research sheds light on the expanding range of the Asian Tiger Mosquito in Europe. It underscores the significant role of holiday road traffic flows, in potentially introducing the mosquito to the Netherlands via the ground vehicle transportation pathway. Furthermore, it highlights high-risk areas within the Netherlands, emphasizing the need for targeted interventions and continued research to comprehensively understand and mitigate the risks associated with the introduction and establishment of the Asian Tiger Mosquito in the Netherlands via this pathway, especially from the currently introduced regions in Belgium.

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1. Introduction

1.1. Background

The Asian Tiger Mosquito, scientifically known as *Aedes albopictus*, is among the most widespread invasive species in the world (Bonizzoni et al., 2013). Originating in Southeast Asia, it has successfully invaded every inhabited continent worldwide during the last 40-50 years (Kraemer et al., 2015). The first sightings of *Aedes albopictus* in Europe occurred in 1979 and 1990 in Albania and Italy respectively (Gossner et al., 2018). Currently, the mosquito has established itself in 13 countries and 337 regions in Europe (ECDC, 2023). This expansion poses a significant threat for human health as the species is a known vector for human arboviral diseases, such as Zika, dengue, chikungunya, and yellow fever (Leta et al., 2018). For instance, Italy reported nearly 300 cases of chikungunya fever in 2007, and France documented the first instances of autochthonous transmitted cases of this disease in 2010, with *Aedes albopictus* identified as the vector responsible for transmitting the virus in both instances (Smith et al., 2016).

The primary way of *Aedes albopictus* global dispersal usually occurs via passive dispersal, which is generally human-mediated and facilitated through various dispersal pathways (Swan et al., 2022). The main passive dispersion channels through which *Aedes albopictus* is introduced into Europe include (1) the trade of used tyres, (2) the trade of Lucky bamboo plant cuttings, (3) vehicles (by ground, sea, and air), and (4) the mosquito's own active natural dispersal. Among these, the global and national transportation of used tyres poses as one of the biggest risks for long-distance importation of mosquitos into and within Europe, as used tyres provide suitable environments for the development of eggs and larval (Ibáñez-Justicia, 2020).

Passive transportation via ground vehicles forms a dispersal pathway that is primarily observed at the national level, although it can extend to continental scales considering contiguous geographical countries, as is the case within Europe (Ibáñez-Justicia, 2020). Over the past two decades, passive transportation dispersal pathways have been recognized as a significant factor contributing to the initial sightings of *Aedes albopictus* in European countries. Research conducted by (Swan et al., 2022) examined the trends in the initial detections of *Aedes albopictus* in European countries from 1940 to 2019. Their study revealed a noteworthy pattern. Since 2000, there has been a substantial increase in the ground vehicle pathway as a mode of introduction for *Aedes albopictus*. This finding suggests that ground vehicles have become a significant factor in the initial detections of this mosquito species in Europe, emphasizing the upcoming importance of this dispersal pathway.

In the Netherlands there is an increase in the numbers of introductions of *Aedes albopictus* sightings in residential areas in the last years (NVWA, 2023) (Schaap, 2023). This trend could be attributed to the rising instances of introductions by citizens traveling within Europe, facilitated by the increasing availability of road networks and a rise in holiday travel activities (referred to as holiday road traffic flow). This combined with the phenomenon of 'hitchhiking,' where *Aedes albopictus* individuals travel with humans in cars, plays a significant role in the mosquito's use of the ground vehicle pathway. This effect is considered a key factor contributing to the expansion of the mosquito (Eritja et al., 2017).

1.2. Problem statement

This growing recognition of the significance of passive dispersal pathways, particularly those involving ground vehicles, aligns with earlier indirect evidence. Reports had long suggested their role in the dispersal of *Aedes albopictus*. However, no systematic study has been conducted due to the challenges of conducting real-time roadside surveys. The first concrete piece of evidence of the role of passive transportation dispersal of *Aedes albopictus* in private ground vehicles in Europe came from a study conducted in Catalonia, Spain (Eritja et al., 2017). This study established that the passive transportation of *Aedes albopictus* with ground vehicles significantly contributes to its dispersion throughout Europe, primarily due to the 'hitchhiking' phenomenon.

Since then, numerous studies have been conducted that elaborated this passive transportation dispersal. For instance, a Swiss study highlighted that together with their observations, the general notion is that *Aedes albopictus* primarily spreads passively via motorized vehicles from Southern regions (Italy, where its established) to the Northern regions (like Germany). This spread occurs, primarily along major road networks like highway route E35 that passes through Switzerland and Germany, connecting Italy to the Netherlands. (Müller et al., 2020). In addition, (Deblauwe et al., 2022) suggested that this type of passive transportation pathway will be become more frequent in the coming years.

Despite the increasing recognition of passive dispersal pathways, particularly those involving ground vehicles, there is a lack of localized studies providing specific evidence to confirm this type of dispersal. However, there have been suggestions that the mosquito may have dispersed itself via ground vehicles in countries such as the Czech Republic (Šebesta et al., 2012), Croatia (Klobučar et al., 2006), France (Roche et al., 2015), and Germany (Pluskota et al., 2008), but these instances lacked concrete supporting evidence. In addition, the discovery of the mosquito's eggs in a trap near the Eurotunnel (Medlock et al., 2017), suggested a potential connection to the use of ground vehicle transportation, although also lacking concrete evidence. Within the Netherlands the use of this dispersal pathway of *Aedes albopictus* also remains mainly unexplored. While comprehensive risk assessments already exist for known pathways in the Netherlands, overseen by the Netherlands Food and Consumer Product Safety Authority (NVWA), this research will provide a more specialized focus. It will concentrate solely on the ground traffic transport pathway, with a specific emphasis on understanding *Aedes albopictus* introduction and distribution through this pathway in case of the Netherlands.

According to modelling predictions, *Aedes albopictus* is projected to be documented in 197 countries by 2080 (Kraemer et al., 2019), indicating a substantial increase from the 86 countries where it has been reported between 1940 and 2020. It is important to gain insight into the dispersal pathways of this mosquito species. This understanding is crucial in countries where it has not yet fully established a presence, such as the Netherlands. Efforts should be concentrated on enhancing the ability to detect this species at points of entry and beyond. Nevertheless, despite the many studies, there are still gaps in our comprehension of *Aedes albopictus* dispersal pathways, including the locations of origin of the individuals utilizing these routes (Swan et al., 2022).

While several studies have already mentioned the potential use of *Aedes albopictus* with this ground vehicle pathway, a tailored analysis is necessary to gain more insight into the mosquito's possible introduction and spread via the ground vehicle pathway to the Netherlands. This research will address these needs, specifically focusing on the unique aspects of *Aedes albopictus* dispersal within Europe and to the Netherlands. To achieve this, Geographic Information Systems (GIS) will play a vital role. GIS offers a powerful tool for analysing and visualizing spatial data, which is essential for comprehending the potential dispersal patterns of *Aedes albopictus* via this pathway.

Previous thesis research has emphasized the significance of GIS in understanding the spread of this mosquito in Europe, particularly highlighting the role of human-assisted pathways, such as ground transport on highways, and demonstrating a likely relationship between highways and the mosquito's distribution (van de Langerijt, 2023). As far as existing literature indicates, no further geospatial analysis is conducted that explores the relationship between ground vehicle pathways and the distribution of *Aedes albopictus*. However, GIS remains commonly employed in research focusing on geospatial risk analysis and forecasting the future distribution of the mosquito, as illustrated in studies such as (Oliveira et al., 2021).

Despite the increasing recognition of passive dispersal pathways, particularly involving the ground vehicle pathway, there is a lack of localized studies providing specific evidence to confirm this type of dispersal, especially in the context of the Netherlands. Additionally, specific locations at risk for the introduction and establishment of *Aedes albopictus* in the Netherlands via this pathway have not been thoroughly identified. This knowledge gap highlights the need for research aimed at interpreting the role of the ground vehicle transportation pathway in the dispersal of *Aedes albopictus* and identifying potential risk zones in the Netherlands.

1.3. Objective and research questions

The objective of this thesis research is to examine the dispersal patterns of *Aedes albopictus* in Europe and identify potential risk zones for its introduction and distribution in the Netherlands. Specifically, the study will focus on investigating the role of passive transportation pathways, particularly ground vehicles on road networks (referred to as road traffic transport), in facilitating the spread of the mosquito from Southern Europe to the Netherlands. Additionally, the research aims to pinpoint specific locations at risk for the establishment of *Aedes albopictus* in the Netherlands. This resulted in the following main research question: "What are the potential locations at risk for the introduction of Asian Tiger Mosquitoes (*Aedes albopictus*) in the Netherlands through ground traffic transport?"

This main research question will be answered through three sub-research questions:

1. How has the range expansion of *Aedes albopictus* in Europe evolved over time, and what key factors can explain these changes?
2. To what extent does holiday road traffic flow on highways connecting the South of Europe and the Netherlands contribute to risk zones for the introduction and distribution of *Aedes albopictus* in the Netherlands?
3. How do road networks in the Netherlands and Europe contribute to the potential introduction and distribution of *Aedes albopictus* in The Netherlands, and what are the high-risk areas?

2. Methodology

2.1. Overall research design

Figure 1 offers a concise overview of the methodology used in this research. It outlines the sequential steps involved in the analysis for each sub-question and shows their connections with each other. Key components such as data input and outputs, literature review, important analysis, and data preprocessing are highlighted, providing a clear depiction of the analytical process flow of the entire research.

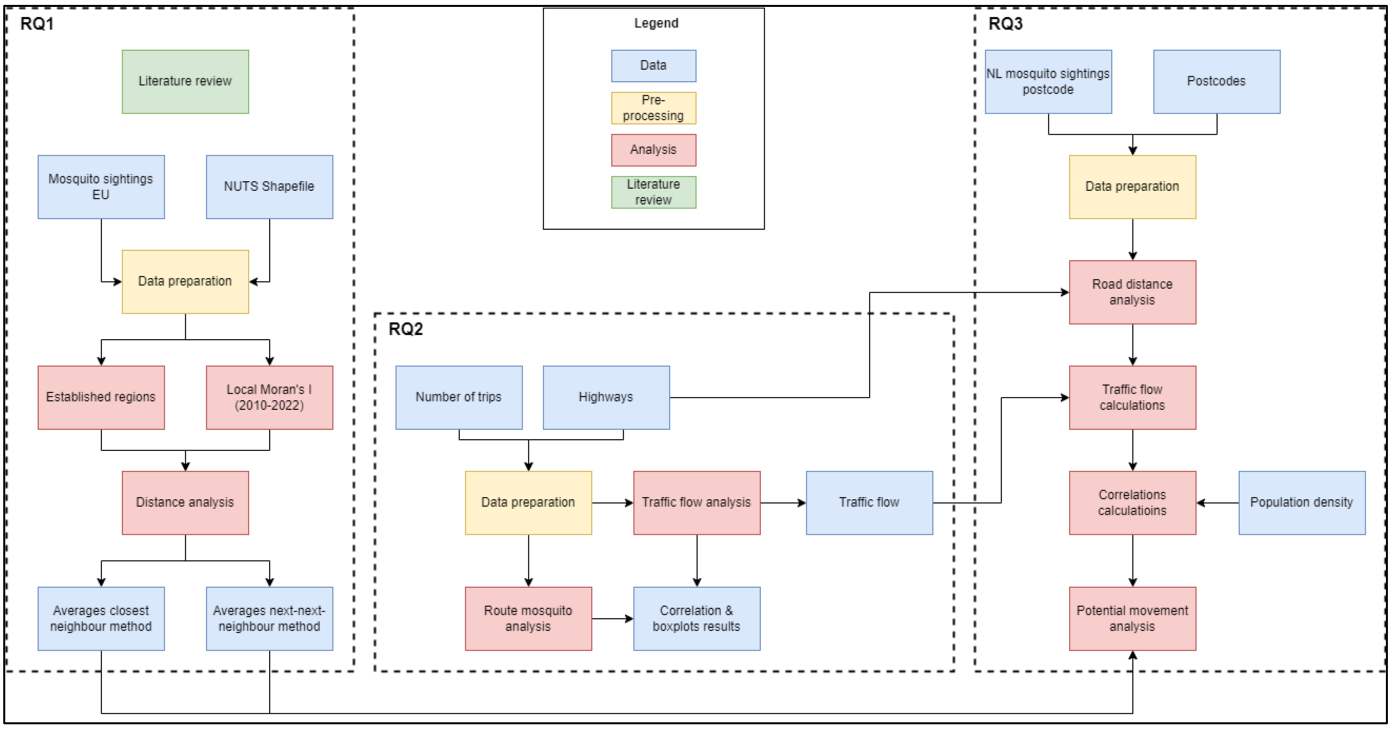

Figure 1: Schematic flow diagram of the overall methodology

The first sub-research question examines the range expansion of *Aedes albopictus* in Europe through a literature review and GIS analysis to calculate the mosquito's average travel distances over time in Europe. The second sub-research question investigates the relationship between holiday road traffic flows and highways along a selected route to the Netherlands using GIS analysis. The third sub-research question identifies high-risk areas for the mosquito's introduction and distribution in the Netherlands via highways through GIS analysis. It utilizes the average distances calculated in the first sub-research question, together with prior results to predict the potential movement of Asian Tiger Mosquito establishment in the Netherlands. The GIS analysis was conducted using the open-source software RStudio version 4.2.2.

2.2. Data sources

Table 1 presents an overview of datasets utilized in the analysis of this research, accompanied by brief descriptions and their respective sources, referencing the origins of the data where it is retrieved from.

Table 1: Overview of the data sources of this research

Source dataset	Description of dataset	Reference
European Centre for Disease Prevention and Control (ECDC)	Tabular dataset of mosquito distributions in Europe	(ECDC, 2023)
Eurostat	Shapefile of all NUTS regions in Europe on four levels	(Eurostat, 2021)
EuroGeographics	GeoJSON files with roads on every selected country	(EuroGeographics, 2017)
Free University of Amsterdam - Faculty of Science	Tabular dataset of number of holiday trips between NUTS level 2 regions in Europe	(Laroche et al., 2023)
The Netherlands Food and Consumer Product Safety Authority (NVWA)	Tabular dataset of postcode-based *Aedes albopictus* findings in the Netherlands	Retrieved from Adolfo Ibáñez-Justicia (NVWA)
Central Bureau of Statistics of the Netherlands (CBS)	Tabular dataset of all postcodes in the Netherlands (only the four numerical digits at the start)	(CBS, 2024)
Central Bureau of Statistics of the Netherlands (CBS)	Tabular dataset of the population density for the Netherlands of persons per square meter on NUTS level 3 scale	(CBS, 2024)

The tabular dataset with mosquito distributions in Europe contains information about the sightings of mosquito species in Europe since 1990 onwards to 2022, and about the classes of establishment of mosquitos: established, introduced, absent, or no data. Spatial explicit data about NUTS regions (Nomenclature of Territorial Units for Statistics) spanning from scale levels 0 to 3 was also used in the research. NUTS organizes EU regions hierarchically, categorizing them by socio-economic significance, with NUTS 1 representing major areas and NUTS 3 denoting smaller specific regions (Eurostat, 2021). The dataset of roads is provided in GeoJSON files. For each selected country separately, values representing roads are retrieved. The dataset of holiday trips will be referred to as ‘Number of Trips.’ It captures the frequency of trips across eight distinct holiday styles, including 261 specific NUTS level 2 regions within the European Union. Each trip is categorized based on its origin and destination region, along with the chosen mode of transportation (air, rail, or road), and specific holiday type. The temporal scope of this dataset covers the years from 2010 to 2018, indicating the average trips per year. In total, the dataset comprises 416,584 individual records, requesting the total amount of holiday traffic flows between the regions (Laroche et al., 2023). The dataset of postcode-based findings encompasses findings spanning from 2016 to 2022, along with corresponding locations and postcodes with four numerical digits and two non-numerical characters, like '1011AB'. The postcodes dataset does only include the four numerical digits, like '1011'. The population density dataset represents the population density of every NUTS level 3 region in the Netherlands.

2.3. Timeframe and study area

The research timeframe spans from 2010 to 2022, determined by the availability of Asian Tiger Mosquito data extracted from the mosquito distribution table of the ECDC. Filtering the table revealed values solely within this timeframe. Values till the year 2022 was included, because the distribution maps upon till the year 2022 were published on the ECDC website, facilitating the possibility of verification of the establishment classifications.

The first sub-research question focusses on all the NUTS level 3 regions in Europe with only a focus on mainland Europe. Overseas European territories were excluded and NUTS level 0 rows representing entire countries and overseas territories were also removed. However, regions on islands were included in this analysis.

The second sub-research question has its focus on a selection of countries, namely the Netherlands, Belgium, France, Spain, and Italy (**Figure 2**). This sub-research question has a focus on a specific route to the Netherlands. The reason behind selecting Spain, France, and Italy is because of their consistent registration and presence of established regions throughout the timeframe of the research, signifying the presence of Asian Tiger Mosquito populations. Italy exhibits a high prevalence of established regions at the start of the research period till the end. France displays an gradual rate of distribution patterns, with Spain displaying a similar distribution over time, with a predominant presence along the Mediterranean coast, shifting more inland in the more recent years. The focus of this sub-research question involves on highways connecting Southern Europe to the Netherlands. Hence, Belgium and the Netherlands are also included. The inclusion of Belgium is essential due to its role in facilitating the passage of highways from Spain, France, and Italy to the Netherlands.

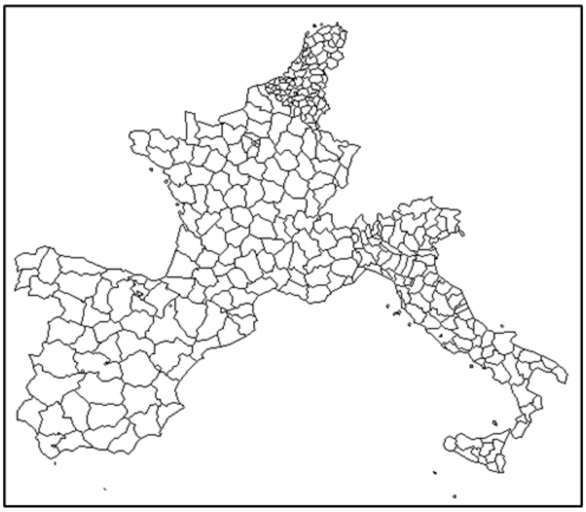

Figure 2: Selection of NUTS level 3 regions for the second sub-research question

On contrast to the first sub-research question, regions on islands within the specified countries have been excluded. The exclusion ensures that the focus remains solely on interconnected highways within the selected countries. The excluded islands are the Balearic Islands in Spain, Sardinia in Italy, and Corsica in France. Sicily, located in close proximity to mainland Italy (approximately 5 km) and accessible by a ferry that accommodates cars frequently throughout the day, remains included in the corresponding analysis.

The third sub-research question focuses exclusively on the Netherlands, utilizing results from the other sub-research questions to identify potential locations at risk for the introduction and distribution of the Asian Tiger Mosquito.

2.4. Range expansion

The aim of this first sub-research question was to determine how the range expansion of *Aedes albopictus* in Europe evolved over time, with taking in consideration possible key factors that could possibly explain these changes. First of all, a small literature review is conducted. After that GIS analysis focusses on calculating the range expansion is executed.

2.4.1. Literature review

To contextualize the results of this first sub-research question within a broader perspective, a literature review was conducted. The aim of this review is to gain insights into the factors influencing the range expansion of *Aedes albopictus* in Europe, with a particular emphasis on understanding ecological and environmental determinants. The search was conducted on Google Scholar, utilizing keywords such as 'Aedes albopictus,' 'habitat preferences,' 'determinants,' 'range expansion,' 'drivers,' 'migration,' 'climate effects,' 'dispersal,' 'human-mediated factors,' 'spread,' and 'urban habitat preferences. These keywords were carefully chosen to explore various facets of *Aedes albopictus* ecology, including its habitat preferences, the determinants of its range expansion, factors driving migration, and the influence of climate and human-mediated elements on its spread. Especially providing new insights that were not touched upon earlier in this research.

Priority was given to recent papers aligning with the specified search criteria. Due to time constraints, only the first ten papers were looked at for each set of combined keywords. Each identified paper underwent an examination of the abstract and conclusion or discussion sections, accompanied by a brief review of other relevant segments. From these selected papers, relevant findings and insights concerning the factors influencing the range expansion of *Aedes albopictus* were extracted and written down.

2.4.2. Data preparation

The data preparation process, presented in the flowchart of **Figure 3**, consisted of a series of systematic steps. It starts by extracting *Aedes albopictus* values from the mosquito distribution dataset of the ECDC, filtering by the value signifying the Asian Tiger Mosquito: 'VECT-000363-A'. Subsequently, only rows where 'isReport' equals zero were retained to avoid a distorted picture of the data. The next step involved filtering for individual years of 2010 to 2022, resulting in dedicated tables for each respective year.

To mitigate duplicate rows, only unique entries from the 'LocationCode' column were preserved. These duplicates originated from the dataset's inconsequential update cycle, resulting in separate indices for region status classifications in the same year but for different months. The next step involved integrating the data layers for each year into the NUTS dataset through a merging process. To narrow the focus to mainland Europe, overseas European territories were excluded and NUTS level 0 rows representing entire countries and overseas territories were removed. Consequently, only NUTS level 3 regions of mainland Europe were retained in the merged layers. For enhanced visualization and analysis, an additional column, 'LegendName,' was created, capturing the classification distribution status of each region based on the 'AssessedDistributionStatus' column, representing the class of establishment: **Table 2**.

Table 2: Creation of 'LegendName' column based on the 'AssessedDistributionStatus' column.

AssessedDistributionStatus	LegendName
INV001A	Established
INV002A	Introduced
INV003A	Absent
INV004A	No Data
INV999A	Unknown

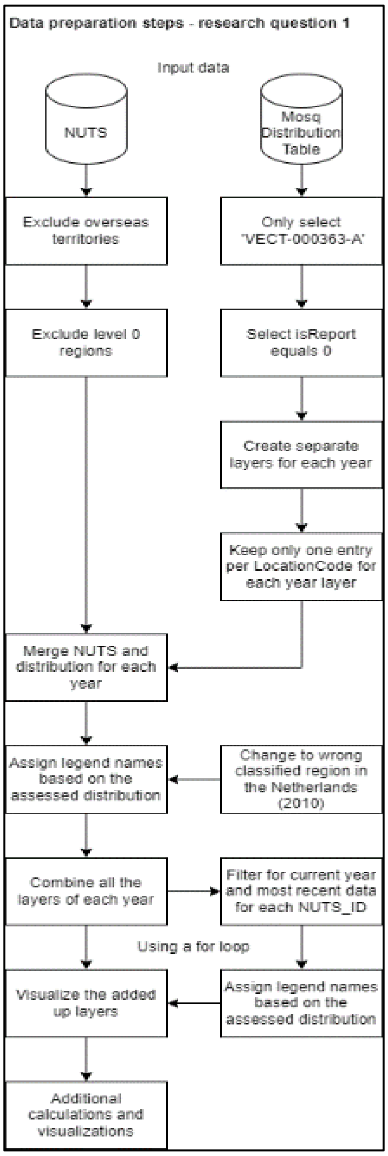

Figure 3: Schematic flowchart of the data preparations steps – sub-research question 1

To combine an added overview across years, the data layers were cumulatively aggregated. A for loop was employed to determine the establishment class of each region for each year iteratively, ensuring that the most recent data for each region was filtered based on the current year. Consequently, regions that maintained their status over several years retained the last assigned status, while regions undergoing changes reflected the altered status for that specific year. Values in the 'LegendName' column were assigned in the same way based on the assessed distribution status. This cumulative approach yielded visualizations representing the aggregated data for each consecutive year from 2010 to 2022. These added aggregated layers facilitated as a visual representation of the distribution over time and as input data for the upcoming analysis.

2.4.3. Local Moran's I definition

To assess the temporal and spatial autocorrelation of *Aedes albopictus* dispersal across Europe, a Local Moran's I analysis is systematically conducted for each year individually, spanning from 2010 to 2022. This analysis is also extended to the added aggregated layers, providing a comprehensive understanding of the Asian Tiger Mosquito's spatial distribution over time. The outcome of this analysis includes hotspot identification for every NUTS level 3 region in each year, highlighting both high and low clusters, as well as high and low outliers—where high values represent significant hotspots.

The identification of spatial outliers and significant hotspot clusters through the Local Moran's I method plays a crucial role in pinpointing priority areas for the mosquito's spatial distribution. This analytical approach aligns with previous research methodologies employed in various studies focused on mosquito-borne diseases in public health contexts. Notable references, such as (Tokarz & Novak, 2018), (Lippi et al., 2020), (Castillo et al., 2011), and (Sugumaran et al., 2009), have utilized the Local Moran's I spatio-temporal cluster analysis to detect hotspots related to diseases carried by endemic mosquito species. In this research, the emphasis is on identifying hotspots specifically for the 'Established' and 'Introduced' regions rather than examining disease – or the total of mosquito presence.

The Moran's I statistic, introduced by Moran in 1950, assesses the global autocorrelation of a variable by considering both the location and values, and can provide an overview of spatial patterns across an entire dataset (Moran, 1950). This Moran statistics yields scores ranging from 0 to 1, where 0 indicates randomness and 1 signifies dispersion. However, due to its global nature, it cannot pinpoint specific areas of significance. To achieve a more localized analysis, a LISA (Local Indicator of Spatial Association) approach is essential, as also mentioned by Tokarz & Novak (2018). Unlike the global statistic, LISA allows for the identification of individual locations' influence on global statistics, facilitating the detection of outliers and hotspots (Anselin, 1995). In this research, the focus is on using a Local Moran's I approach with LISA to identify statistically significant hotspot regions with spatial outliers, enabling a comparison with neighbouring regions and facilitating visualization. This local approach is more tailored to the specific needs of this research as compared to the global approach.

2.4.4. Local Moran's I analysis

Figure 4 illustrates a flowchart with the sequential steps involved for executing the Local Moran's I analysis. This was done for the separate years' layers and the added aggregated layers. The analysis utilizes two key input data tables generated during the data preparation phase: the NUTS table containing only mainland European regions and the specific NUTS table for each respective year. The process begun by filtering the mainland NUTS table to retain only NUTS level 3 regions. Subsequently, this filtered table is merged with the NUTS table corresponding to the specific input year as inserted into the function.

To facilitate the Local Moran's I analysis, which requires numerical data, a conversion is necessary, as the initial data includes categorical values, specifically the 'LegendName' column derived from the 'AssessedDistributionStatus' column (**Table 2**). To focus on regions with confirmed data and to detect spatial autocorrelation with significant hotspots and spatial outliers, priority is given to regions classified as 'Established' and 'Introduced.' These categories are assigned numerical values for the analysis, with 'Established' regions assigned the value 2 (indicating the highest importance as regions where the Asian Tiger Mosquito is established) and with 'Introduced' regions assigned the value 1 (indicating regions where the species is introduced without confirmed establishment). All other regions are assigned the numerical value 0, signifying the absence of mosquito presence or the categorization as 'Unknown' or 'No Data.' These numerical values are incorporated into a new column named 'LegendCode.'

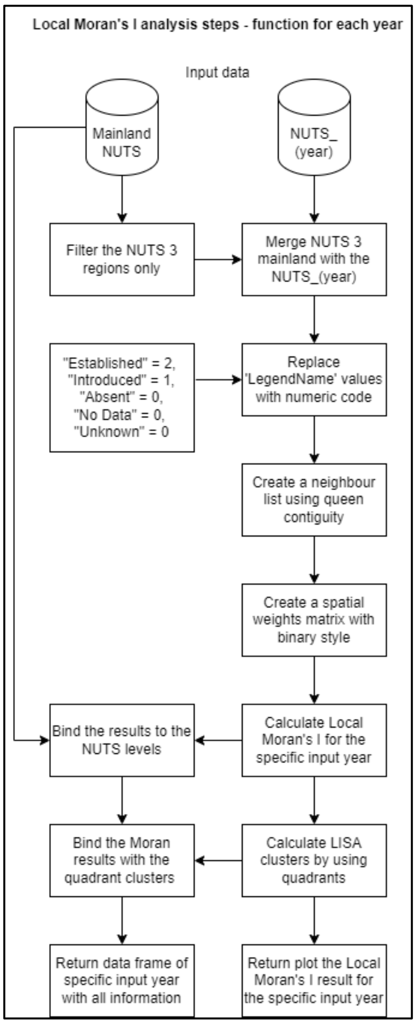

Figure 4: Schematic flowchart of the Local Moran's I analysis

With the adjusted table, a neighbourhood list is constructed using queen contiguity, identifying regions that share a common border. This generated a list of neighbouring regions for each specific region. Subsequently, a spatial weights matrix is created with a binary style ('B'), setting 'zero.policy' to true. This ensures spatial relationships are treated as either connected or not, accommodating regions with zero neighbours, such as islands. If 'zero.policy' is set to false, the spatial matrix deletes regions with 0 neighbours, and an error will occur, as the spatial weight matrix must have the same number of rows (NUTS regions) as the input NUTS table of a specific year. After addressing missing values in the table by setting them to zero, the spatial weights matrix and neighbourhood list enable the calculation of Local Moran's I analysis for each region in the specific input year. These results are then integrated with NUTS input data for mainland Europe, and further combined with the calculated LISA clusters.

Before computing LISA clusters, the Local Moran's I values are centred around their mean to standardize the outcomes. LISA clusters are determined by initially establishing a significance threshold of 0.1, a common value for identifying statistically significant clusters. Subsequently, a data quadrant is constructed based on standardized values of the variables of interest in the 'LegendCode' column, the standardized Local Moran's I value, and the significance threshold. This quadrant categorizes regions as Low-Low (1), Low-High (2), High-Low (3), High-High (4), or Insignificant (0).

2.4.5. Distance analysis

Figure 5 shows the flowchart with the steps taken in the distance analysis. The distance analysis was executed twice. The first analysis utilized the result layers of the Local Moran's I analysis with the added aggregated layers of the distribution (added Moran results). The second analysis solely involved the added aggregated layers of the distribution. For the added Moran results, the focus was on regions exhibiting High-High and High-Low spatial patterns, identified as quadrants 3 and 4. In this context, specific columns were selected and renamed for clarity. Additionally, a new column labelled 'Year' was introduced to associate each hotspot region classification row with the respective year. In the case of the distribution layers, the analysis concentrated on regions labelled as 'Established' in the 'LegendName' column. A 'Year' column was also added in this case to signify the corresponding year for each established region.

Following the previous steps for both analyses, a combined table was generated from the standalone tables of each year. This integration resulted in a unified data frame of the hotspot regions or established regions along with their respective years. Within this table, for each row representing a specific region, the x and y coordinates were computed to derive centroids. These centroids, signifying the precise midpoint of each region, were subsequently stored in a newly created column named 'Centroid.'

The Euclidean distances between centroid points of regions, illustrating the geographical span in kilometres from a specific region in one year to another region's centroid in the subsequent year were calculated. The process involves computing distances for pairs of consecutive years (e.g., 2012 to 2013, 2013 to 2014, and so forth, up to 2021 to 2022), providing a nuanced understanding of small changes between years. For each year pair, a distinct distance matrix is calculated. In this matrix, each value denotes the distance between the centroid of a hotspot – or established region in the initial year and the centroid of a hotspot- or established region in the subsequent year. Rows in these distance matrices correspond to the initial year, while columns represent the regions of the following year. The regions of both years are identified by their respective NUTS code, as recorded in the 'NUTS_ID' column of the input data of this function.

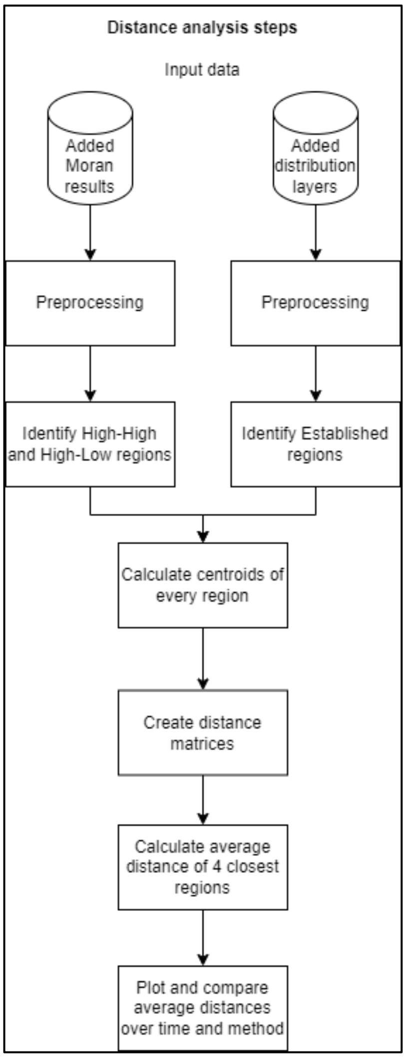

Figure 5: Schematic flowchart of the distance analysis

After the creation of distance matrices for each pair of consecutive years, the next step involved calculating the average distances for each region in each distance matrix. This calculation was specifically performed for the four closest regions in distance of a specific region. The selection of four closest regions was based on the average number of neighbours for the regions in the input datasets. In the combined layer of the added Local Moran's I results, the average amount of neighbours is 4.1, and for the established layers it is 3.9. To avoid potential bias in the results, same regions already included in the distance matrix were excluded from the closest regions. They were represented in the distance matrices as zero. This could happen when a region was a hotspot – or an established region in the initial year and was also classified as it in the previous year. The reason behind focusing on the closest regions was to mitigate the impact of potential outliers. For instance, considering distances from regions in Switzerland to regions in Italy could significantly differ from distances between regions in Switzerland and Turkey. Utilizing the average of the four closest distances per region aimed to provide a more meaningful representation of the mosquito's distribution or movement, emphasizing relationships with neighbouring regions rather than those located far away.

This process of calculating the closest average distances (referred to as closest neighbour method from now on), was executed for every region in the rows of the distance matrices, representing the initial years from which distances were calculated. Subsequently, for each year pair, the average distance was computed, allowing for the calculation of the overall average distance across all year pairs. This approach aimed to capture nuanced changes in the distribution of the Asian Tiger Mosquito while minimizing the impact of outliers. At last, the temporal distribution trends were depicted graphically, allowing for a direct comparison between the Local Moran's I results approach and the established regions approach. This comparison included an examination of the overall average distance calculated through each method.

The distance analysis was also extended to a national level, using France as an illustrative example. The decision to include national-level analysis was driven by the variation in the size of NUTS level 3 regions across European countries, as is shown in **Figure 6**. For instance, regions in Germany differ significantly in size compared to those in Spain. The selection of France was intentional, considering its interesting established distribution pattern over time. In 2010, only a few regions in the South were established, but over time the establishment of regions progressed, reaching almost to halfway up the country, to the height of Paris. By conducting the analysis at the national level, it serves as a demonstration of how the approach of this distance analyses can be applied, offering a more detailed and specific overview of country-level dynamics.

Figure 6: NUTS level 3 regions across Europe

In the distance analysis, another slight variation was introduced in the calculation of average distances by considering an additional set of neighbours, referred to as the 'next-next-neighbour' method in this research. This involves the inclusion of one extra neighbour along with the existing four closest regions in distance from a specific region. For each of these four closest regions, the nearest neighbour of these four regions is also incorporated into the average distance calculation. This 'next-next-neighbour' example methodology provides a more comprehensive understanding, acknowledging the potential movement of Asian Tiger Mosquitos across multiple NUTS regions per year, rather than limiting it to only the four closest neighbouring regions in a given year-pair.

2.5. Holiday road traffic flows on highways

The aim of this second sub-research question was to determine the extent of holiday road traffic flow on highways connecting the South of Europe and the Netherlands on contributing to risk zones for the introduction and distribution of *Aedes albopictus* in the Netherlands. This involved GIS analysis focusses on determining the relationship between holiday road traffic flows to the Netherlands on highways and the mosquito establishment classes.

2.5.1. Data preparation

Highways

For each selected country in this second sub-research question, a road table is acquired in form of a GeoJSON file. The highways for each country are identified based on the 'rtn' column, representing the route name. For example, in the case of the Netherlands, highways are identified by names starting with the letter 'A', so the dataset is filtered to include only those roads. The same process is repeated for other countries, where the highways are identified by specific naming conventions, with only those highways being extracted from the 'rtn' column (**Table 3**). Following this step, the highway data from each country is combined into a single data frame, which will be utilized for subsequent analyses.

Table 3: Filtered values from the 'rtn' column to retrieve solely the highways for each country

Country	'rtn' column filter
Belgium	Values beginning with 'A' or 'R' with maximum of one digit
France	Values beginning with 'A'
Italy	Values beginning with 'A' or 'R'
the Netherlands	Values beginning with 'A'
Spain	Values beginning with 'A-,' 'AP-,' or 'R' with maximum of 2 digits

Additionally, to focus on the regions relevant to each selected country, the NUTS data frame is filtered accordingly. This ensures that only the regions related to the specific country, identified by the 'CNTR_CODE' column, are included in subsequent analyses. Following these steps, the highway data from each country, along with the corresponding filtered regions of each selected country, is combined into a new data frame (**Figure 7**).

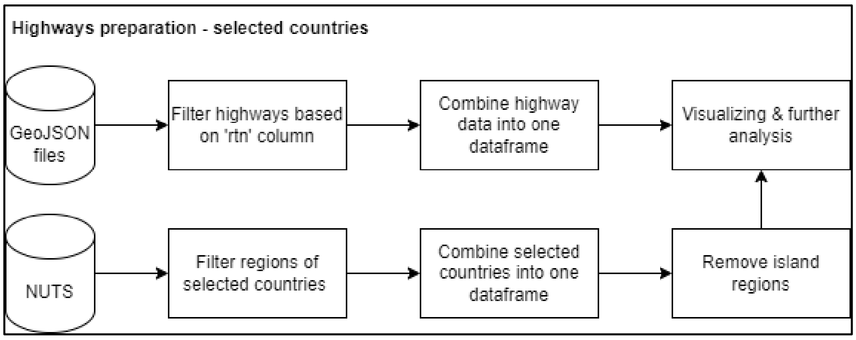

Figure 7: Schematic flowchart of the highways data preparations of the sub-research question 2

Number of Trips

This dataset focuses on holiday traffic flows associated with eight distinct holiday styles. Within this research, all holiday types will be treated uniformly as they all are presumed holiday traffic flows. The flowchart of the data preparation for the 'Number of Trips' dataset is shown in **Figure 8**. Unnecessary columns are removed, with saving the 'Road' column. This process resulted in multiple rows containing identical combinations of 'FROM' and 'TO' pairs, because of the deletion of all holiday styles. To rectify this redundancy, the dataset is grouped based on the 'FROM' and 'TO' columns. Within each group defined by unique combinations of 'FROM' and 'TO', the total value of the 'Road' column is calculated. This aggregation ensures that only one unique combination of 'FROM' and 'TO' exists, combining multiple rows into a single row for each distinct pair of regions. Furthermore, the dataset initially utilized outdated NUTS level 2 codes for French regions. To correct this, an adjustment of the incorrect French codes to the correct ones was performed using an Excel file sourced from the European Commission. This way, the incorrect French codes were replaced with the accurate NUTS codes, aligning the dataset with the correct regional codes.

The dataset was further refined to specifically target trips destined for the Netherlands. This involved filtering the dataset based on values corresponding to the Netherlands in the 'isocntry_TO' column, which signifies the destination country. Subsequently, the selected countries were filtered out from the 'isocntry_FROM' column, representing the origin countries. This process ensured that only trips originating from the selected countries to the Netherlands as the destination country were included in the dataset.

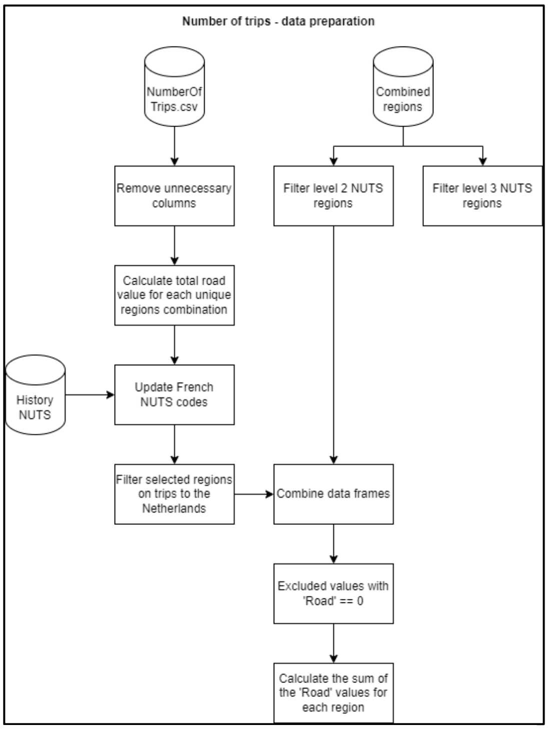

Figure 8: Schematic flowchart of the 'Number of Trips' data preparations of the sub-research question 2

The combined regions dataset was filtered to get the NUTS levels 2 and 3 regions, resulting in two distinct data frames. Following this, the data frame specifying the destination as the Netherlands is combined with the filtered NUTS level 2 regions dataset. This combination generated a new data frame focusing on regions with the destination as the Netherlands, with the geometry added. Additionally, rows containing the value 0 in the 'Road' column, were excluded. This ensured that the analysis concentrates solely on regions with more than zero road traffic destined for the Netherlands. Lastly, the total amount (sum) of the 'Road' column for each unique region in the 'FROM' value was calculated to determine the total number of road traffic flow for each region of the selected countries with the Netherlands as destination.

2.5.2. Route mosquito analysis

The flowchart of the methodology of this analysis is shown in **Figure 9**. The analysis focusses on determining the total length of highways within each NUTS level 3 region across the chosen countries. This process began by filtering the NUTS level 3 regions from the combined dataset of selected countries, which was prepared in the data preparation stage. Following this, the islands that were determined to be excluded from the dataset were removed. To pinpoint the total amount of highways within the selected regions of the chosen countries, a spatial intersection was employed. This allowed for the calculation of the lengths of highways within each region, achieved through summing up each highway segment. The data was then grouped by 'NUTS_ID' since, at this stage, the highway data still retained smaller segments. The lengths of the highways were derived from the geometry of each highway segment. Subsequently, the highway data was combined with the corresponding 'NUTS_ID' values, resulting in the creation of a data frame encompassing the total highway length in kilometres for each region.

In the following steps, distinct data frames were generated for each year within the time range of 2010 to 2022, corresponding to distinct categories of Asian Tiger Mosquito establishment: established, introduced, and absent. The 'absent' category encompassed values labelled as 'No Data' and 'Absent,' assuming no prevalence in both classes. These data frames were then filtered based on the added up aggregated regions data frames of each year, created in the first sub-research question. For each year, the filtering was applied to the chosen regions and their respective category, resulting in the creation of specific data frames such as 'established_year,' 'introduced_year,' and 'absent_year.' Subsequently, these data frames were combined with the previously generated data frame containing information on the total highway length in kilometres for each region. This process ensured the availability of data frames for each combination of categories and years, integrated with details on highway lengths for each region and their corresponding geometries.

The next step involved counting the regions with highways by looping through the previously generated data frames for each category and year. Within this process, a new column named 'Has_Highways' was created to store information on whether a region possesses highways. The assignment in this column is binary, with a value of 0 denoting the absence of highways and 1 indicating the presence of highways in a region. After that, the proportion of regions with highways for each specific category and corresponding year is calculated. This computation involved dividing the sum of the 'Has_Highways' column by the total number of rows in the data frame, which corresponds to the overall count of regions. The outcome is a proportion expressed as a value ranging from 0 to 100, representing percentages. These calculated proportions are then stored in dedicated vectors.

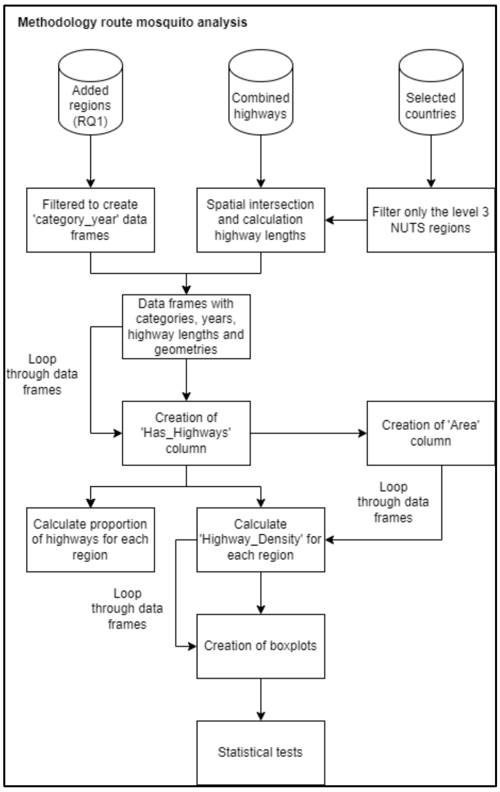

Figure 9: Schematic flowchart of the methodology of the route mosquito analysis

Following this the highway density for every region within each category and corresponding year could be computed. The binary classification system overlooks distinctions in highway length quantities among regions. A loop was implemented to iterate through each data frame, calculating the area in square kilometres for every region and adding a new column named 'Area.' Subsequently, the highway density is determined for each region by dividing the total highway length by its respective area. The results are then stored in a new column labelled 'Highway_Density.' This column signifies the length in kilometres of highways per square kilometre in a specific region. For example, a value of ≈ 0.24 indicates that there are approximately 0.24 kilometres of highways for every square kilometre in that particular region.

Furthermore, distinct maps are created for each category and year, displaying the evolving highway densities over time. These visual representations offer insights into how highway densities change for each region category throughout the specified years. Examining these maps provide a clear view of the temporal patterns in highway densities and their relationship with the distribution of the categories and regions. The visualizations depict the dynamic interplay between highway densities, the classification categories, and regions across the time period.

With the information about the highway density of each region, boxplots for comparing different establishment classes with highway densities over the years could be constructed. A loop iterates through the data frames associated with each category and corresponding year, specifically examining the 'Highway_Density' column. For every year, the boxplots are generated, illustrating the distribution of highway density among the three categories. The box plots provide information on key statistics such as the median, as well as the presence of outliers for each year and category. The median and outliers of every year are extracted, and used for identifying patterns, differences, and facilitating further visualizations.

The extracted medians from each category and year's boxplot were subjected to statistical tests to assess the significance of differences in highway density medians. Initially, a Shapiro-Wilk Test was employed to verify the normal distribution of the data. Following this, Levene's Test was conducted to examine the homogeneity of variances, ensuring equality of variances across all years. Subsequently, a Friedman Test, a non-parametric test for repeated measures of ANOVA, was performed to check whether significant differences exist among the medians of the three categories throughout all the years. After this, the Nemenyi Post-hoc Test was executed to pinpoint specific pairs of groups displaying significant differences in median values, considering the entire dataset. Finally, the Wilcoxon Signed-Rank Test was employed to check and compare median differences between consecutive years for each year and category individually.

2.5.3. Traffic flow analysis

The flowchart of the methodology of this analysis is shown in **Figure 10**. Initially, the analysis involved converting the NUTS level 2 region codes from the 'Number of Trips' cleaned dataset into NUTS level 3 region codes. This is achieved by establishing a conversion table that links each NUTS level 3 region with its corresponding NUTS level 2 regions. Subsequently, this conversion table is merged with the 'Number of Trips' dataset to provide a more aggregated perspective of the data. The NUTS level 3 regions are stored in the respective columns ‘TO_3’ and ‘FROM_3’. This dataset now included all the NUTS level 3 regions which destination to the Netherlands with an origin region being a NUTS level 3 region of the selected countries.

Using this data frame, the overall road traffic flow from the NUTS level 3 regions of the selected countries to the Netherlands could be computed. Specifically, the analysis excludes regions in the Netherlands to shift the focus towards traffic flow to the Netherlands rather than within it. This involved aggregating the unique regions in the 'TO_3' column to determine the total incoming road traffic flow to the regions within the Netherlands from regions outside the Netherlands. The same was done for the ‘FROM_3’ column to determine the total road traffic flow from each region outside the Netherlands directed towards the Netherlands.

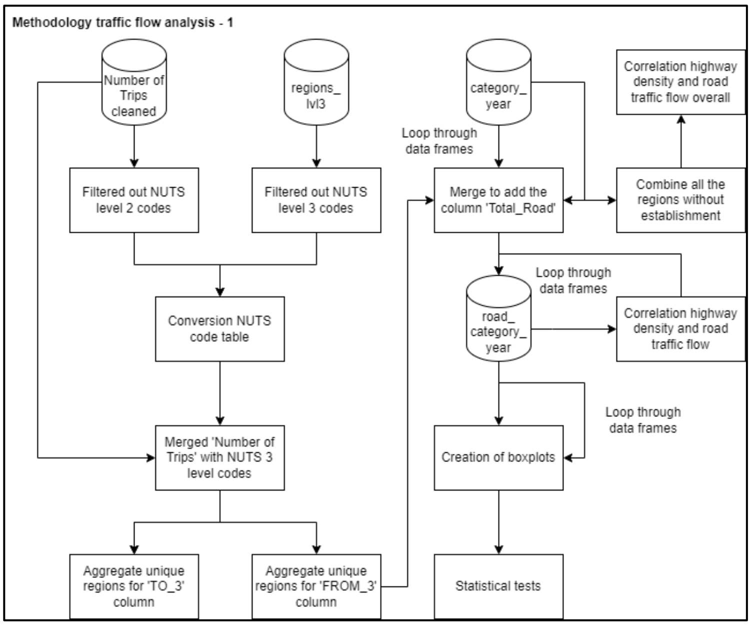

Figure 10: Schematic flowchart of the first part of the methodology of the traffic flow analysis

Subsequently, the summarized 'FROM_3' data frame was merged with each 'category_year' data frame generated in the route mosquito analysis. This process aimed to add the total road traffic flow data into a new column named 'Total_Road' for each establishment category. For every region within a specific category and year, the cumulative road traffic flow to the Netherlands was included. These new data frames were called like ‘road_category_year.’ Regions absent from the 'Number of Trips' dataset or lacking road traffic flow data to the Netherlands were assigned a value of 0 in this context. The regions of the Netherlands were removed from each of the new 'category_year' data frames because, as previously stated, they will not be considered. Subsequently, any NA values in the 'Total_Road' column of each data frame were replaced with 0.

Following this, a comparison between highway density and road traffic flow was conducted for each year and category using correlation analysis. Initially, the correlation was calculated across all regions without considering the establishment classes (overall). Subsequently, the correlation between highway density and road traffic flow was assessed within each category and corresponding year. The results were then aggregated into a new data frame for further analysis and visualization.

With the information about the road traffic flow for each region in each category and corresponding year, boxplots for comparing different establishment classes with road traffic flow over the years could be constructed. A loop iterates through the data frames associated with each category and corresponding year, specifically examining the 'Total_Road' column. For every year, the boxplots are generated, illustrating the distribution of highway density among the three categories. The median and outliers were then assessed using the same statistic tests as in the route mosquito analysis.

After this, the analysis switches it focus to the distribution of road traffic flow from established regions within the selected countries, particularly considering traffic directed towards regions within these countries. The flowchart of the part of the methodology of this analysis is shown in **Figure 11**. Initially, the destinations of road traffic flow were examined across all regions within the selected countries. This involved creating a new dataset comprising all regions as destination instead of solely the destination as the Netherlands. Their respective NUTS level 3 region codes and associated road traffic flow values were also included. Subsequently, this data was aggregated based on the 'TO_3' column to determine the overall road traffic flow originating from each region towards other regions within the selected countries. This created a data frame encompassing the total incoming road traffic flow for every NUTS level 3 region within the selected countries. With this the total road traffic flow to each country could be calculated.

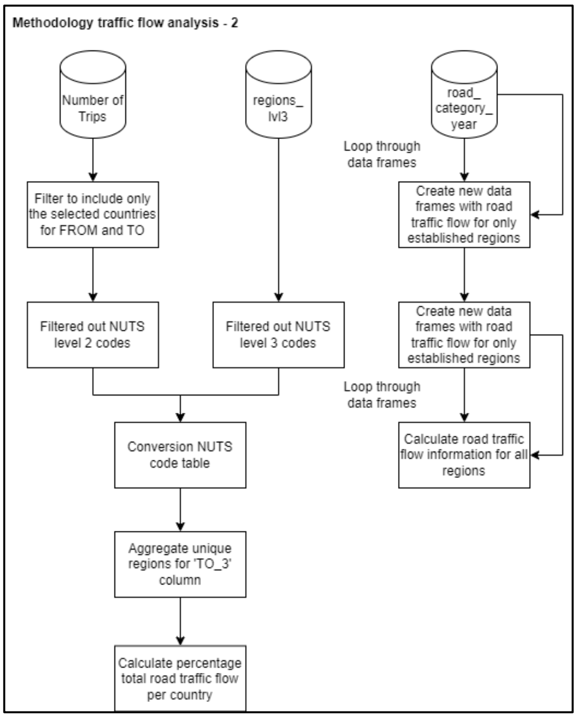

Figure 11: Schematic flowchart of the second part of the methodology of the traffic flow analysis

Next, new data frames were created named 'NumberOfTrips_selected_year' using the 'road_category_year' data frames. These new data frames contain road traffic flow data for established regions for each year. Subsequently, the total traffic flow and the percentage of road traffic flow for each country from each established region is calculated. These operations are performed in a loop, resulting in data frames that show the total road traffic flow at the NUTS level 3 region of the selected countries from each established region for each year. Additionally, it calculates the total road traffic flow from all established regions for each year, along with the percentages of the incoming total road traffic flow from each established region aggregated at the country level for each year. Additionally, this was also done for the introduced – and absent regions.

2.6. Identifying high-risk areas

The aim of this third sub-research question was to determine how road networks in the Netherlands and Europe contribute to the potential introduction and distribution of *Aedes albopictus* in The Netherlands, with pinpointing possible high-risk areas. This involved GIS analysis focusses on distances from Asian Tiger Mosquito findings to highways, incoming holiday road traffic calculations, correlation calculations, and a prediction analysis on the potential establishment in the Netherlands.

2.6.1. Road traffic correlations analysis

The flowchart of this analysis is illustrated in **Figure 12**. The postcode-based Asian Tiger Mosquito findings dataset from the NVWA was combined with the postcode dataset from CBS. After combining, each postcode findings locations included geometries. To retrieve the geometries for each region in the population density dataset, it was merged with one of the previously used data frames.

An intersection was conducted between the highways in the Netherlands, as used in the second sub-research question, and the postcodes associated with the Asian Tiger Mosquito findings. The objective was to filter out only those postcodes with findings that intersected with highways. Subsequently, the distance from each unique postcode (all postcodes with findings) to the nearest (Euclidean distance) highway was computed to determine their proximity to highways. This enabled the calculation of the average distance to highways for all postcodes with findings.

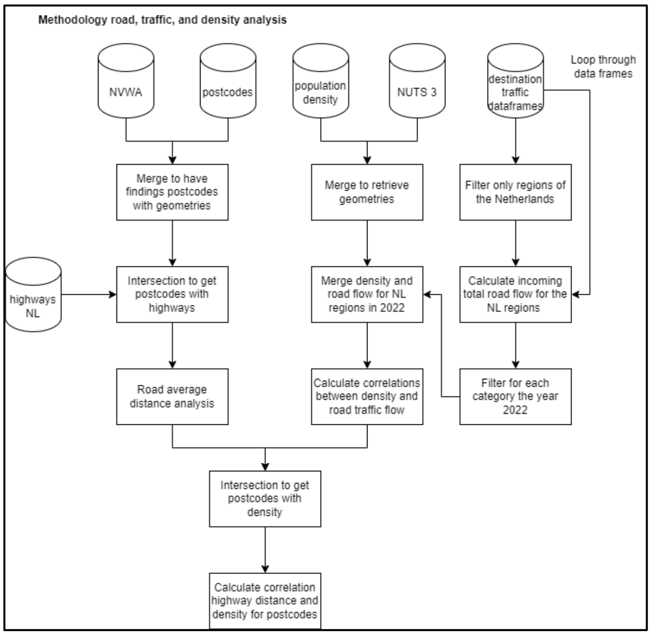

Figure 12: Schematic flowchart of the methodology of the road traffic correlations analysis

The total amount of holiday road traffic flow from the selected countries to each region in the Netherlands was calculated, for each establishment category. The data frames used for this were constructed for each category and year in the second sub-research question, containing information about holiday road traffic flow from specific categories to regions within the selected countries. Subsequently, the data was filtered to include only regions within the Netherlands. Using a loop over each corresponding data frame for each year and category, the total holiday road traffic flow for each year and category received by regions in the Netherlands was computed. Following this, maps of the Netherlands were generated for the year 2022, illustrating the total holiday road traffic flow received by each region in the country for each category. This analysis specifically focused on the year 2022, being the most recent year within the research timeframe, allowing for comparison with population density data from the same year later on.

Subsequently, the relationship between holiday road traffic flow for each category and population density was examined, focusing solely on the year 2022. Population density data was incorporated into the previously created datasets by adding a column representing population density for each region within the Netherlands. Correlations between total holiday road traffic flow and population density were assessed for all three categories. Additionally, to determine the density of each postcode with findings, an intersection was performed to match each postcode with its corresponding population density value at NUTS level 3 scale. Consequently, the correlation between highway distance and population density of each postcode with findings could be determined.

2.6.2. Potential movement analysis

The aim of this analysis was to predict the years regions in the Netherlands could potentially become established, based on the average ranges of distribution determined in the first sub-research question. The flowchart is shown in **Figure 13**. Those averages were found to be approximately 353.76 km per year for the 'next-next-neighbour' method and 84.15 km per year for the closest neighbour method.

Initially, the distribution data frame for 2022 was filtered to include only regions in the Netherlands. Subsequently, centroids were calculated for each region. Following this, only the established regions were filtered out. The same was done for the other selected countries. Using a loop, the closest established region centroid was determined for each region in the Netherlands. Distances from each centroid of a region in the Netherlands to the closest established region centroid in the selected countries were calculated in kilometres. Next, each region's distance was divided by the respective average range of distribution to estimate the time in years it might take for that region to become established. These values were calculated up from the year 2022 and added to the existing data frame. This was done two times, creating two prediction maps.

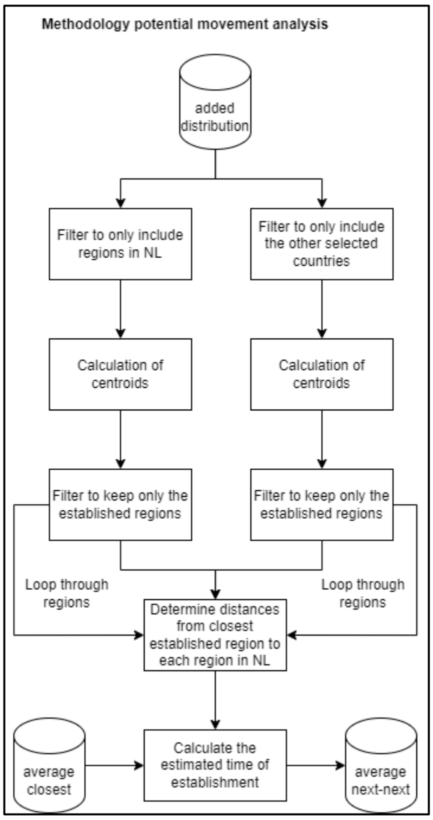

Figure 13: Schematic flowchart of the methodology of the potential movement analysis

3. Results

This chapter will present the results for each sub-research question separately, starting from the first and concluding with the third.

3.1. Range expansion

The aim of this first sub-research question was to determine how the range expansion of *Aedes albopictus* in Europe evolved over time, with taking in consideration possible key factors that could possibly explain these changes. First of all, the results of the literature review are given. After that, the results of the GIS analysis focusses on the range expansion will be presented.

3.1.1. Literature review

The literature review aims to provide a comprehensive understanding of *Aedes albopictus* ecology, encompassing habitat preferences, range expansion determinants, migration drivers, and the influences of climate and human-mediated factors. The following texts offer insights from relevant studies, shedding light on key aspects of the species' behaviour and distribution.

Aedes albopictus exhibits diverse habitat preferences and a capacity for adaptation in different regions. A study in Southern Texas indicated a greater abundance of the mosquito near coastal areas (Champion & Vitek, 2014). The species is adaptable to diverse environments, including artificial and natural breeding sites such as used tires, abandoned containers, and abandoned car pieces (Simard et al., 2005). In Southwestern Virginia, *Aedes albopictus* demonstrated higher abundance in open residential areas, displaying its adaptability to diverse landscapes. (Barker et al., 2003). A study in Saint Louis, USA, indicated higher mosquito densities in human-dominated areas, reflecting the species' thriving in metropolitan regions (Westby et al., 2021). Additionally, *Aedes albopictus* is likely to prefer rural, suburban, and vegetated urban areas, as was found in a study conducted in Rio de Janeiro state, Brazil, and Florida, USA (Braks et al., 2003).

Understanding the determinants of *Aedes albopictus* range expansion is fundamental for anticipating its future spread. Crucial to understanding the determinants of *Aedes albopictus* range expansion is knowledge of key limiting factors in Europe. Presently, these include winter temperatures in Eastern Europe and summer temperatures in Southern Europe (Cunze et al., 2016). Projections for the future indicate a potential shift, with low winter temperatures persisting in Eastern Europe while other factors become more significant in Central Europe. Accessibility, absolute humidity, and annual minimum temperatures emerge as strong predictors for *Aedes albopictus* presence (Dickens et al., 2018)(Roiz et al., 2011). Predictions point towards Europe becoming more suitable for *Aedes albopictus* as climate change progresses (Laporta et al., 2023). The temperature of the environment is a critical factor influencing the life of insects, including *Aedes albopictus*. As poikilotherms, their body temperature is not uniform, and they depend on different strategies to minimize the risk of thermal stress, influencing their distribution rates. So, the increasing temperatures will have an impact on the distribution rates of *Aedes albopictus* (Reinhold et al., 2018). The potential for the mosquito invasion into new locations correlates closely with their ability to adapt to changing climate conditions (Liu et al., 2019).

Human activities play an important role in *Aedes albopictus* dispersion, with land use influencing mosquito establishment. Observations in France highlighted the difficulties associated with sporadic movements from areas far away of already established regions to new locations. This underscores the challenges involved in initiating new invasion fronts (Roche et al., 2015). Urbanization substantially increases the mosquito density, larval development rate, and adult survival time of *Aedes albopictus*, leading to higher densities in pupae and adult populations in urban areas compared to suburban and rural areas (Li et al., 2014). Anthropogenic factors exhibit stronger correlations with the potential range of the mosquito as compared to climate factors (Dickens et al., 2018). However, further investigation is needed to determine the respective impacts of anthropogenic and climate factors on the potential range distribution of *Aedes albopictus* (Nie & Feng, 2023). This fact is also supported by the results of this literature review, which highlights a lack of information concerning the potential influencing factors on the mosquito's range expansion.

3.1.2. Established regions

In Europe in total up until the year 2022, 362 NUTS level 3 regions are classified as established, 137 regions as introduced, 742 regions as absent, and 215 regions as having no available data (see **Figure 14**). Notably, most of the established regions are concentrated in Italy, totalling 107 regions. In [GIF 1]() the total distribution over time is depicted.

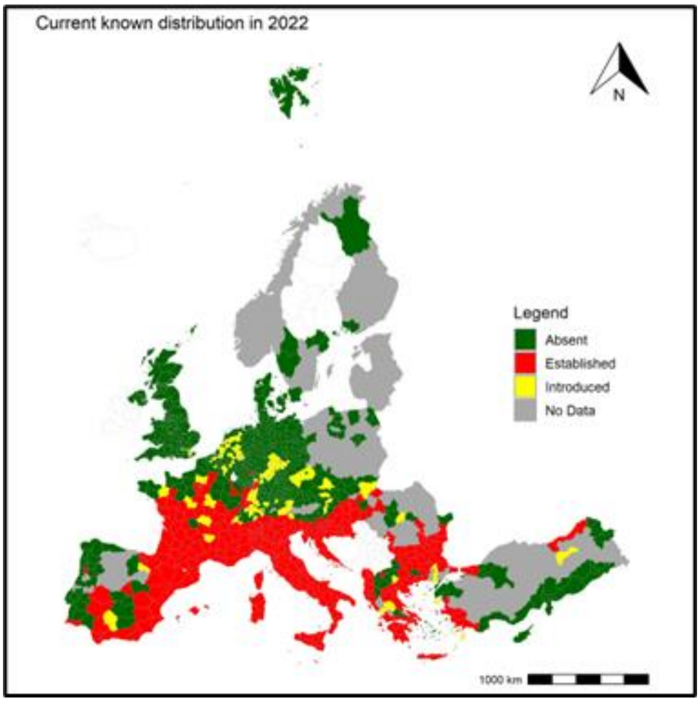

Figure 14: Current known distribution of Aedes albopictus in Europe (situation 2022)

A region is labelled as 'Established' if there is evidence of a breeding and overwintering mosquito population in at least one municipality within a certain region. 'Introduced' status applies when the mosquito has been introduced in the region within the last five years of the distribution status date, without confirmed establishment. 'Absent' indicates that field studies or surveys were conducted, but no introduction in the last five years was reported, or no established population was found. 'No Data' signifies the absence of data for at least the last five years, and 'Unknown' indicates a lack of information about field observations on the mosquito during the last five years (ECDC, 2023).

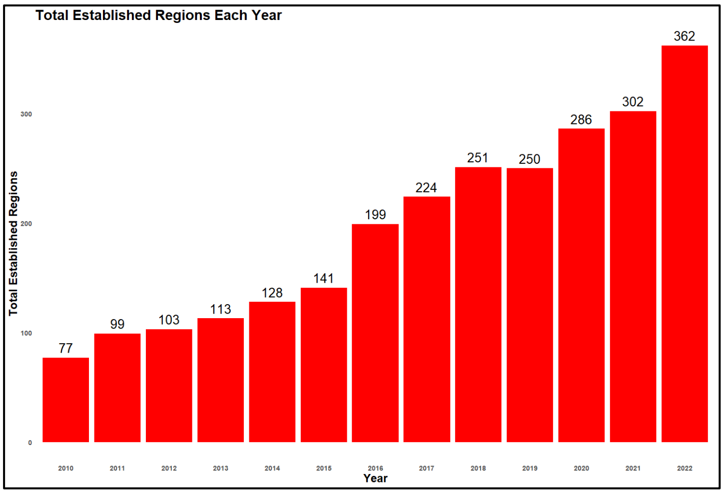

Figure 15: Total count of established regions each year (2010-2022)

Figure 15 reveals a continuous upward trend in the total regions that are classified as established each year. It shows the expanding range of *Aedes albopictus* over time in Europe, with significantly a larger number of regions classified as established, from 77 in 2010 to 362 in 2022. This trend underscores the increasing prevalence of *Aedes albopictus* across European regions.

3.1.3. Local Moran's I

Comparing the Local Moran's I result maps of each year (GIF 2) to the currently known distribution, a similar distribution pattern is observed. Regions classified as High-High and High-Low are primarily found in the Southern part of Europe along the Mediterranean Sea. This same pattern can also be seen in the current known distribution map for the established regions (**Figure 14**).

The LISA clusters are related to the distribution patterns of *Aedes albopictus*. High-High regions indicate clusters with consistently established or introduced populations over time. High-Low suggests regions where the presence is significant but surrounded by regions with less prevalence. Low-Low regions are characterized by a consistently low presence, surrounded by similar regions. Low-High points to regions with a lower presence surrounded by regions with higher prevalence. Insignificant regions lack a clear spatial pattern, indicating no significant similarity or dissimilarity with neighbouring regions.

3.1.4. Distance analysis

The distance analysis was executed two times. First, with the results of the Local Moran's I analysis, focusing exclusively on the regions that were classified as High-High and High-Low in all the years (referred to as hotspots). Secondly, solely with regions classified as 'Established' in all the years. **Figure 16** displays the maps of the centroids for both approaches.

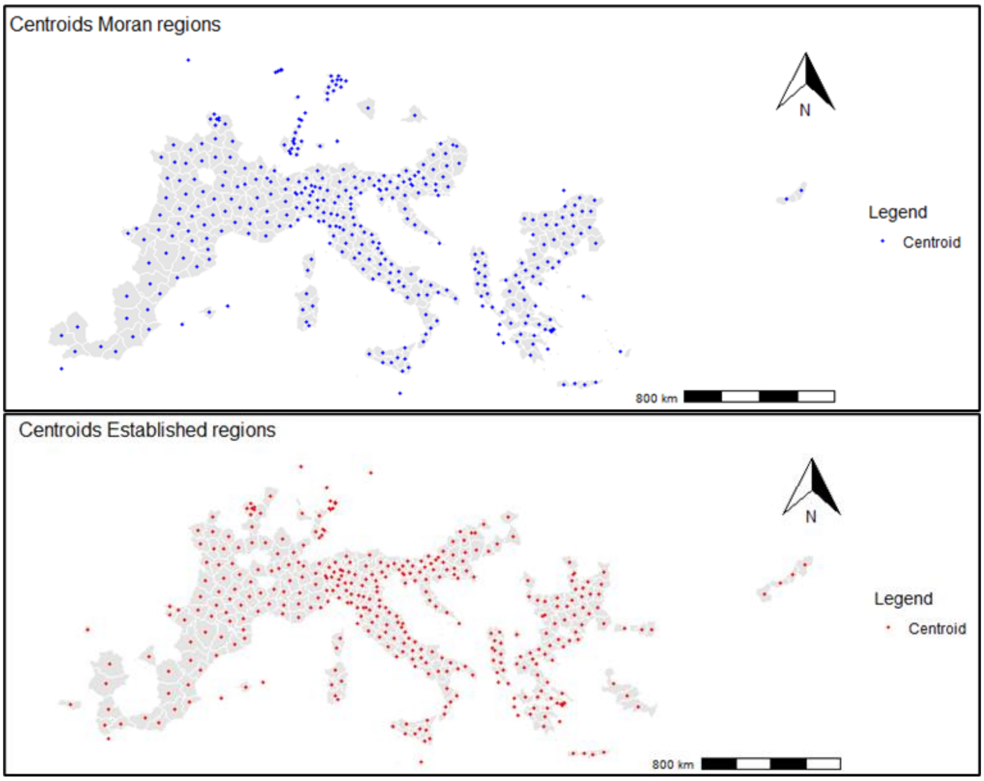

Figure 16: Centroid maps for the Local Moran's I regions classified as High-High or High-Low in all the years (above) and regions classified as established in all the years (below)

With centroids assigned to each region, a set of twelve distance matrices was created for each year-pair from 2010-2011 towards 2021-2022. These matrices depict the distances, measured in kilometres, between regions classified as hotspots or established in a given year and those in the subsequent year. If a region retains the same classification in both years, the distance is denoted as 0. However, when a region is not classified as such in the previous year, the distance matrices do not exhibit uniform row and column numbers. **Table 4** provides an example illustrating a subset of a distance matrix, featuring regions in Albania and Bulgaria classified as hotspots or established in both years, along with corresponding distance values in kilometres.

Table 4: Subset of a distance matrix – displaying distances between regions in Albania and Bulgaria in a given year-pair

	AL011	**AL012**	**BG312**	**BG341**
AL011	0.00	53.90	323.64	594.73
AL012	53.90	0.00	373.75	648.35
BG312	323.64	373.75	0.00	351.32
BG341	594.73	648.35	351.32	0.00

Table 5 and **Table 6** presents the averages for the distance matrices of each year-pair, derived either from the Local Moran's I hotspots or solely from the established regions.

Table 5: Average distances for every year-pair - Local Moran's I regions (left)
Table 6: Average distances for every year-pair – established regions (right)

Year-pair	Average distance
2010-2011	79.94
2011-2012	71.07
2012-2013	71.29
2013-2014	79.51
2014-2015	74.29
2015-2016	75.01
2016-2017	70.67
2017-2018	82.12
2018-2019	77.36
2019-2020	77.83
2020-2021	80.49
2021-2022	70.97

Year-pair	Average distance
2010-2011	97.56
2011-2012	86.006
2012-2013	86.91
2013-2014	83.84
2014-2015	80.002
2015-2016	75.83
2016-2017	82.98
2017-2018	84.66
2018-2019	88.48
2019-2020	80.50
2020-2021	85.28
2021-2022	77.78

The average distances of the Local Moran's I hotspots consistently appear lower than from the year-pairs of established regions (**Table 7**). The smallest difference is observed in 2015-2016, with only a 0.83 km difference. The largest difference is observed in the 2010-2011, exhibiting a gap of 17.63 km.

Table 7: Difference between the average distances of the two approaches

Year-Pair	Difference
2010-2011	17.63
2011-2012	14.94
2012-2013	15.61
2013-2014	4.326
2014-2015	5.70
2015-2016	0.83
2016-2017	12.31
2017-2018	2.55
2018-2019	11.12
2019-2020	2.67
2020-2021	4.79
2021-2022	6.81

The average distance across all year-pairs for the Local Moran's I hotspots is 75.88 km, whereas the average distance for the established regions is 84.15 km. The difference between the overall averages is 8.27 km. This is over a 13-year time period, involving twelve two-year pairs. **Figure 17** shows the average distances of the mosquito's movement of the two approaches compared with each other in a line graph. The dashed line represents the trendline, which is declining slowly when the years go on.

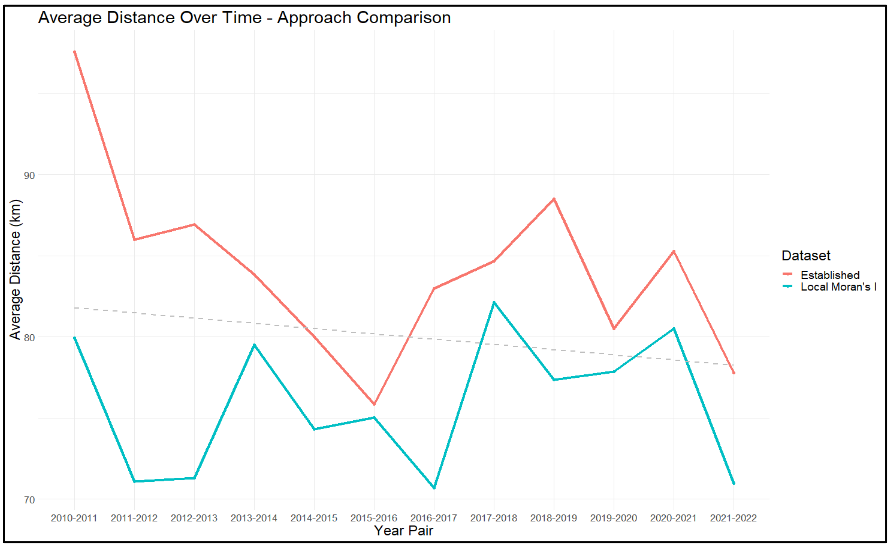

Figure 17: Average distances in kilometres over time for the two approaches: hotspots (Local Moran's I) in blue & the established regions in red

The variation in average distances between the two approaches can be attributed to the fact that the established regions approach uses only established regions. In contrast, the Local Moran's I hotspots approach does not include all established regions, as they might not be classified as High-High or High-Low regions in each year, because not all established regions are in close proximity to the other regions. This is illustrated in **GIF 3** for the Local Moran's I and **GIF 4** for the established regions with showing the average distances from each of those regions to other regions close by.

The distance analysis was also performed on national level, here for France. The average distance of the mosquito's movement in this case across all year-pairs for the Local Moran's I hotspots is 113.80 km, whereas the average distance for the established regions is 136.35 km. The difference between the overall averages is 22.55 km. This is over a 13-year time period, involving twelve two-year pairs. **Figure 18** presents the average distances on continental and national scales compared with each other.

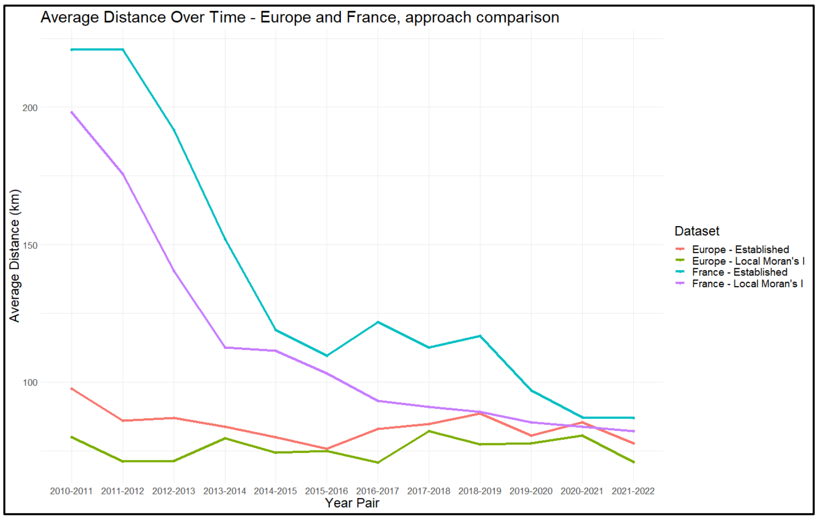

Figure 18: Average distances in kilometres over time on national (France) and continental (Europe) scales

The average distance over time at the national level of France is notably higher than the average distance calculated for Europe as a whole. This distinction is particularly apparent in the earlier years. However, as time progresses, the distances gradually decrease, and in the most recent years, the average distances from both approaches and scales come together, reaching approximately the same average distance. Those differences in the average distances for France can be explained by GIF 5 for the Local Moran's I and GIF 6 for the established regions with showing the average distance from each of those regions to other regions close by.

The distance analysis was also executed with the so called 'next-next-neighbour' method, with incorporating one extra neighbour to each of the four closest neighbours where the average distance was additionally calculated on. With this method the average mosquito's movement distance across all year-pairs for the Local Moran's I hotspots is 335.46 km, whereas the average distance for the established regions is 353.76 km. The difference between the overall averages is 18.31 km. This is over a 13-year time period, involving twelve two-year pairs. In **Figure 19** the average distances with the 'next-next-neighbour' method are compared with each other in a line graph. The dashed line represents the trendline, which is increasing when the years go on.

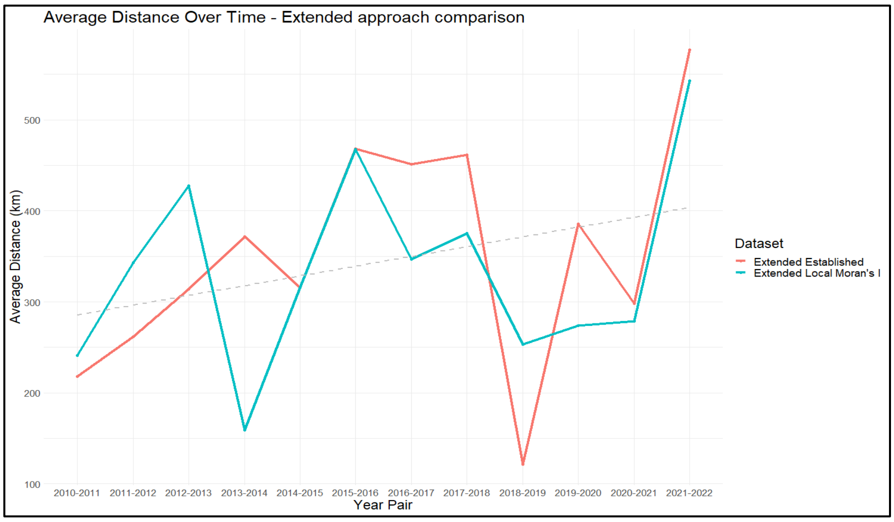

Figure 19: Average distances in kilometres over time using the 'next-next-neighbour' method for the two approaches: hotspots (Local Moran's I) in blue & the established regions in red

Table 8: Average distances for every year-pair - Local Moran's I regions 'next-next-neighbour' method (left)
Table 9: Average distances for every year-pair – established regions 'next-next-neighbour' method (right)

Year-Pair	Average Distance
2010-2011	241.02
2011-2012	343.39
2012-2013	427.51
2013-2014	159.33
2014-2015	315.97
2015-2016	467.39
2016-2017	346.84
2017-2018	375.45
2018-2019	252.99
2019-2020	273.66
2020-2021	279.06
2021-2022	542.87

Year-Pair	Average Distance
2010-2011	218.16
2011-2012	261.67
2012-2013	314.23
2013-2014	371.85
2014-2015	315.80
2015-2016	468.53
2016-2017	451.09
2017-2018	461.30
2018-2019	121.27
2019-2020	385.92
2020-2021	298.38
2021-2022	576.95

Table 8 and **Table 9** presents the averages for the distance matrices of each year-pair for the 'next-next-neighbour' method, derived either from the Local Moran's I hotspots or solely from the established regions. The average distances calculated from the year-pairs of the Local Moran's I hotspots vary between the years when compared to the established regions (**Table 10**).

Table 10: Difference between the average distances of the two approaches – 'next-next-neighbour' method

Year-Pair	Difference
2010-2011	-22.86
2011-2012	-81.72
2012-2013	-113.28
2013-2014	212.52
2014-2015	-0.16
2015-2016	1.13
2016-2017	104.25
2017-2018	85.85
2018-2019	-131.72
2019-2020	112.26
2020-2021	19.32
2021-2022	34.08

In the year-pair 2014-2015, the smallest difference of approximately -0.16 km suggests that, for this specific period, the average distance is close between the two approaches. Negative values indicate that, for the specific year-pairs, the established region approach yielded a smaller average distance compared to the Local Moran's I approach. In the year-pair 2013-2014, the largest difference is of 212.52 km. Here, the Local Moran's I approach produced a smaller average distance.

3.2. Holiday road traffic flows on highways

The aim of this second sub-research question was to determine the extent of holiday road traffic flow on highways connecting the South of Europe and the Netherlands on contributing to risk zones for the introduction and distribution of *Aedes albopictus* in the Netherlands. The results of the GIS analysis will be presented, beginning with the relationship between highways and the mosquito establishment classes. Following this, the role of holiday road traffic flows to the Netherlands with regards to the establishment classes will be presented.

3.2.1. Route mosquito analysis

Table 11: Total highway lengths (km) of every selected country

Country	Total highway length (km)
Belgium	1869
France	11480
Italy	7022
the Netherlands	2487
Spain	12165

In **Table 11** the total highway length in kilometres is given for each of the selected countries. The presence of highways varies among the NUTS level 3 regions in the selected countries (Spain, France, Italy, Belgium, and the Netherlands). Italy has twelve out of 107 regions (11.21%) which are lacking highways. In Spain, a mere two out of the fifty-two regions (3.85%) lack highways. France, with ninety-six regions, has six regions (6.25%) without highways. Belgium follows, with four out of its forty-four regions (9.09%) having no highways. Lastly, the Netherlands, with forty regions, records only two regions (5%) without highways.

The total length of highways in each NUTS level 3 region of the selected countries is visually presented in **Figure 20**. Notably, NUTS level 3 regions in Spain are the largest, contributing to their higher total highway lengths. France, with comparatively larger regions than the Netherlands and Belgium, shows fewer regions with highways. In contrast, the Netherlands displays a more concentrated distribution of highways despite having smaller regions. As a result, it has lower highway lengths at region level.

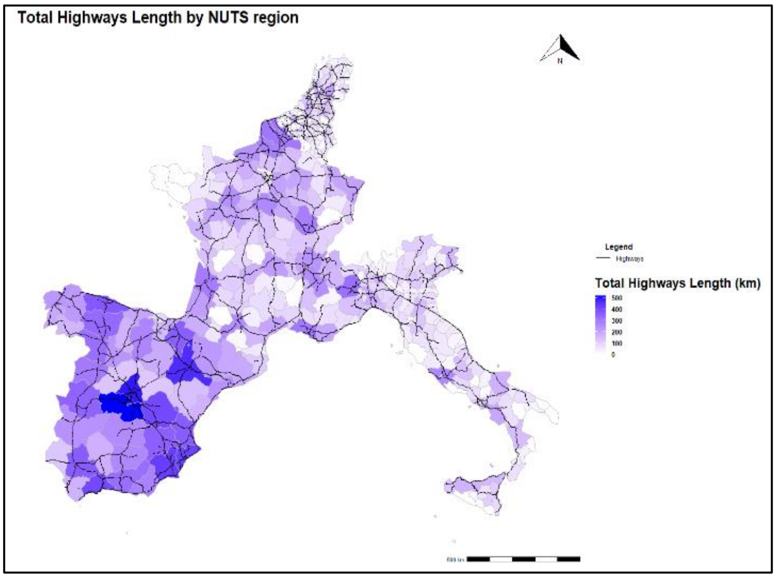

Figure 20: Total highways length for each region of the selected countries

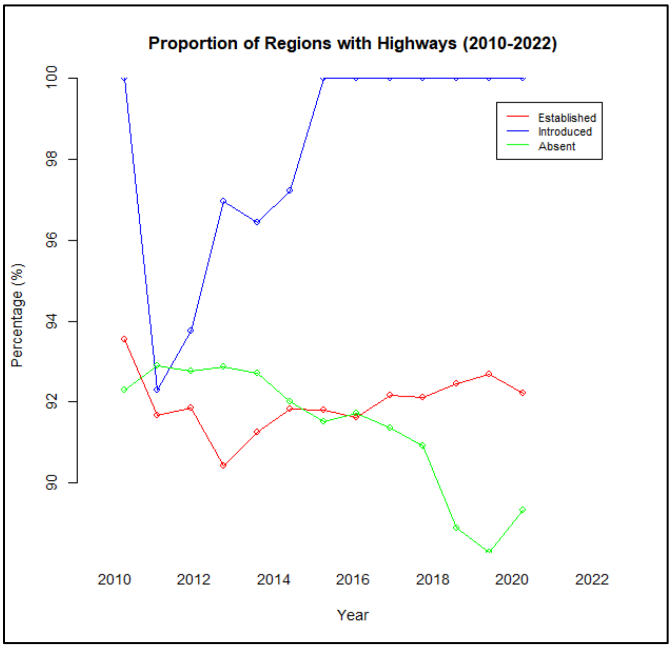

Figure 21: Proportion of regions with highways per establishment category throughout the years

Out of the total 329 NUTS level 3 regions, 26 regions are identified as lacking highways. This corresponds to an approximate percentage of 92% of regions with highways. **Figure 21** illustrates the percentage of regions with highways for each year and category. The introduced regions initially all had highways, reaching 100% in 2010. Subsequently, there was a temporary decline in 2011, followed by a steady increase. Since 2016, all introduced regions consistently had highways. Conversely, the absent category experienced its highest percentage of regions with highways from 2011 to 2014. However, a declining trend is observed in more recent years, falling below 90%. The established category, while exhibiting a slight decline in the earlier years, has maintained a relatively stable pattern in recent years. Approximately 92% of established regions consistently feature highways. On average, introduced regions consistently exhibit a high highway occurrence throughout the years, with a mean of 98.2%. In contrast, absent regions show the lowest mean of 91.34%, while established regions show highway presence with an average of 91.69%.

Figure 22 shows the total highway densities for each region. Belgium and the Netherlands stand out with the highest highway densities compared to the other countries. The Netherlands exhibits an average of 0.07 kilometres of highways per square kilometre, while Belgium follows closely with an average of 0.06. In contrast, Italy has an average of 0.0295, France 0.027, and Spain 0.028 kilometres of highways per square kilometre.

Boxplots were generated for each year spanning from 2010 to 2022. Each boxplot provides a visual representation of how highway densities differ among regions within each establishment category for a specific year. For every year the boxplots are given in GIF 7. Over the years, the median of highway densities in established regions appears to be relatively stable, experiencing only minor fluctuations over time. Meanwhile, absent regions exhibit a similar pattern, but with slightly more fluctuations. In case of introduced regions, the median starts slightly higher than other categories and undergoes fluctuations, with a notable increase in recent years compared to the others. Outliers are predominantly observed in absent regions during the earlier years, while still having a fair amount in recent years. Established regions show a gradual increase in outliers over time. Introduced regions mostly display outliers from 2013 to 2018.

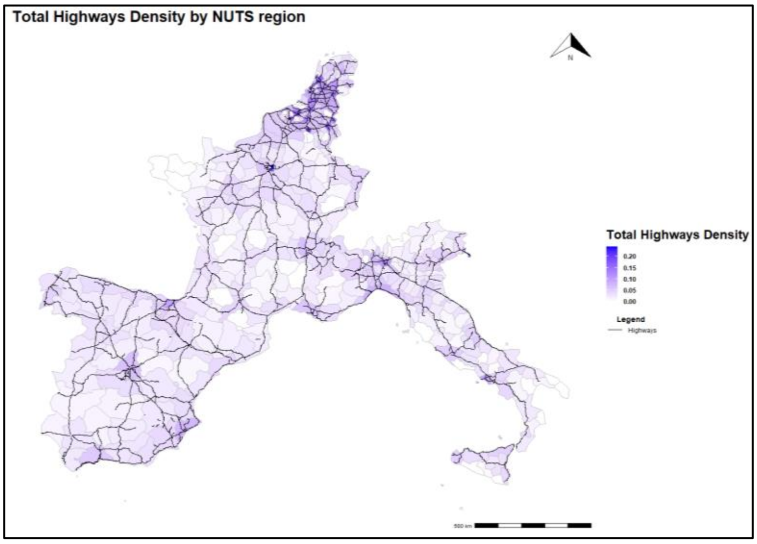

Figure 22: Total highway density for each region of the selected countries

As depicted in **Figure 23**, the median of the introduced regions starts at a higher median value than the other categories but makes a rapid decline. Notably, from 2016 to 2019, absent regions surpass the other categories in median values. However, starting in 2019, introduced regions exhibit a substantial increase. Between 2010 and 2012, the established category had higher medians than the absent category, but in the years after, it declined slowly, becoming the lowest median value. Despite this gradual decline, it remained relatively stable for the remainder of the time period.

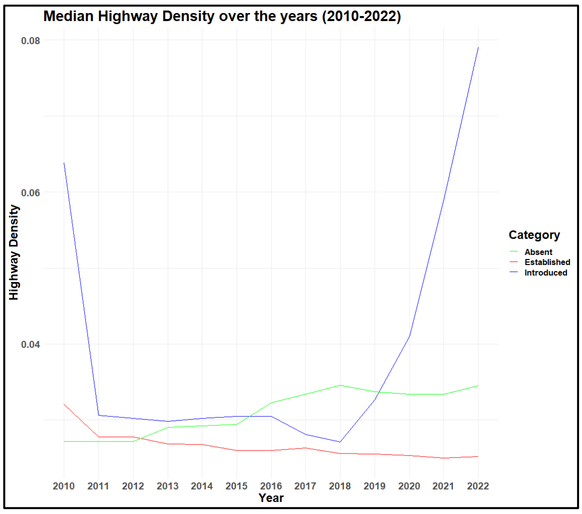

Figure 23: Median highway densities for each establishment category over time

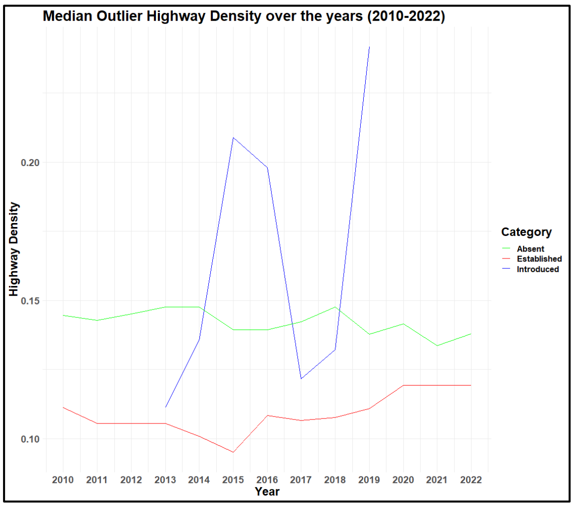

Figure 24: Median outlier highway densities for each establishment category over time

Figure 24 illustrates the presence of the outliers. Both the absent and established categories exhibit a similar trend, with the absent outliers consistently being higher than the established outliers. The introduced region outliers show more variability. They are initially absent in the early years, experiencing a notable increase in the subsequent years, undergoing a big decline in 2017, followed by a great increase from 2018 to 2019, and finally, no outliers are observed in the years after that.

Maps were generated for the variations in the distribution of highway densities for each category. These spatial distributions are shown for the established category in GIF 8, for the introduced category in GIF 9, and for the absent category in GIF 10.

Statistical tests were conducted to test the differences in medians between the categories for each year. The Shapiro-Wilk Test for normality provided a p-value of 2.474e-09, indicating no normality in the data. Subsequently, Levene's Test was employed to test for homogeneity in variances, resulting in a p-value of 0.04728, slightly below the 0.05 significance level, suggesting a lack of homogeneity in variances. Given the non-normality and non-homogeneity in the data, the Friedman Test was determined suitable for the data. The Friedman Test yielded a p-value of 0.0009119, which is lower than the significance level. This implies that significant differences exist in the medians of the establishment categories across the years. To pinpoint these differences, a Nemenyi post-hoc Test was conducted. The detailed results of the Nemenyi post-hoc Test are presented in **Table 12**.

Table 12: Nemenyi post-hoc Test results – highway density

	Absent	Established
Established	0.07870	-
Introduced	0.25917	0.00057

In addition, a Wilcoxon Test was executed to assess specific paired comparisons between consecutive years for each category. The results of the Wilcoxon Test across all combined years indicated consistently high p-values, all close to 1. These high p-values suggest a lack of evidence to reject the null hypothesis, implying that there is no significant difference in the distributions of values within each category between consecutive years.

3.2.2. Traffic flow analysis

Out of the total 84 NUTS level 2 regions from the selected countries, the 'Number of Trips' dataset identified 49 unique regions. These forty-nine regions are depicted in **Figure 25**, illustrating the total holiday road traffic flow from each region to the Netherlands. Notably, regions in Belgium exhibit the highest overall road traffic flow to the Netherlands on average annually between 2010 and 2018. Additionally, certain regions in France also demonstrate relatively high traffic volumes. While road traffic flow to the Netherlands appears to be lowest from Italy and Spain.

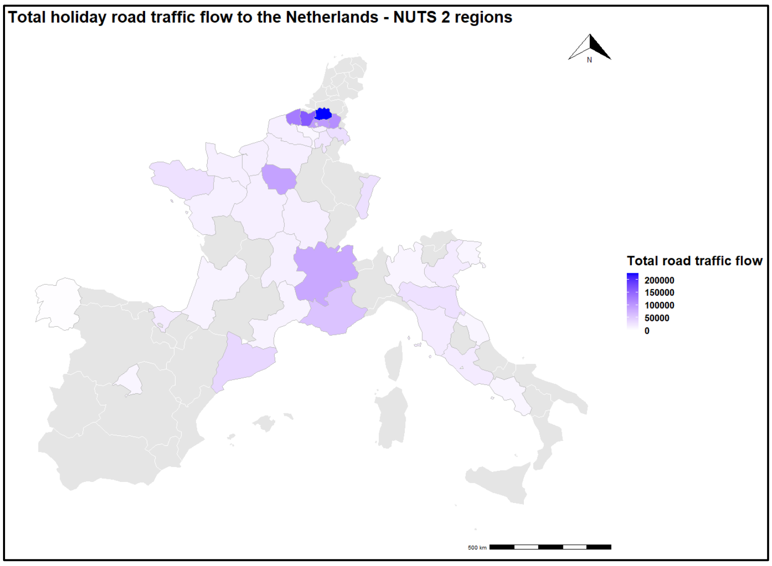

Figure 25: Total holiday road traffic flow to the Netherlands from NUTS 2 level regions of the selected countries

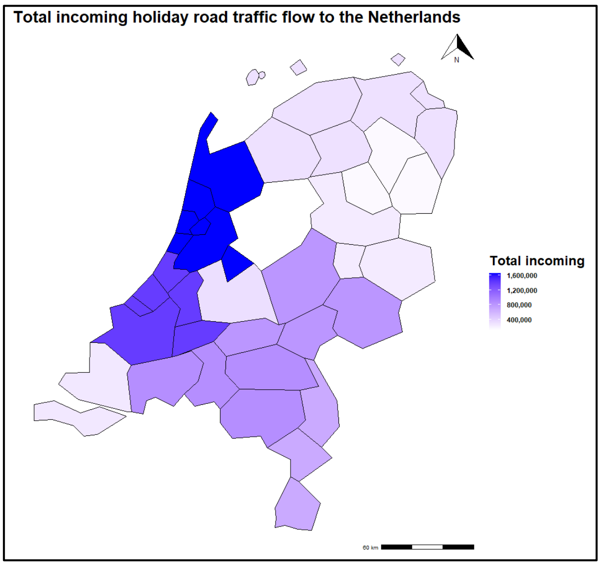

Figure 26: Total incoming holiday road traffic flow to the Netherlands – from the selected countries

Figure 26 depicts the average annual total holiday road traffic flow from the selected countries to the Netherlands between 2010 and 2018. The incoming holiday road traffic flow to the NUTS level 3 regions of the Netherlands varies from approximately 200,000 to 1.6 million. Notably, Noord-Holland and Zuid-Holland exhibit the highest traffic volumes, while the province of Drenthe experiences the lowest road traffic. Additionally, the Southern provinces of the Netherlands generally receive a relatively higher total incoming road traffic from the selected countries compared to the provinces in the North.

A correlation analysis was conducted for the relationship between highway density and incoming holiday road traffic flow into the Netherlands. The resulting correlation coefficient is 0.38, suggesting a weak positive correlation between highway density and total road traffic flow destined for the Netherlands. This implies that regions with higher highway densities tend to have slightly higher levels of road traffic flowing into the Netherlands.

In **Figure 27** the correlation coefficient results for each category and corresponding year are given. In the absent category, there is a correlation coefficient of approximately 0.42 during the earlier years. However, this correlation weakens gradually over time, with coefficients declining to below 0.3 in more recent years. This signifies a decreasing positive correlation between highway density and road traffic flow from absent regions to the Netherlands. For the established category, there is a slight negative correlation coefficient observed in the initial years, followed by an increase to around 0.3 in the latest year. Despite this increase, the relationship between road traffic flow from established regions to the Netherlands and highway density remains negligible. Conversely, the introduced category demonstrates notable variations. Initially, a negative correlation is observed, hovering around -0.35 in the earlier years. However, there is a substantial increase in correlation coefficient values in subsequent years, reaching as high as 0.82 in 2020. This indicates a strong positive correlation between road traffic flow from introduced regions to the Netherlands and highway density in these areas, particularly in recent years.

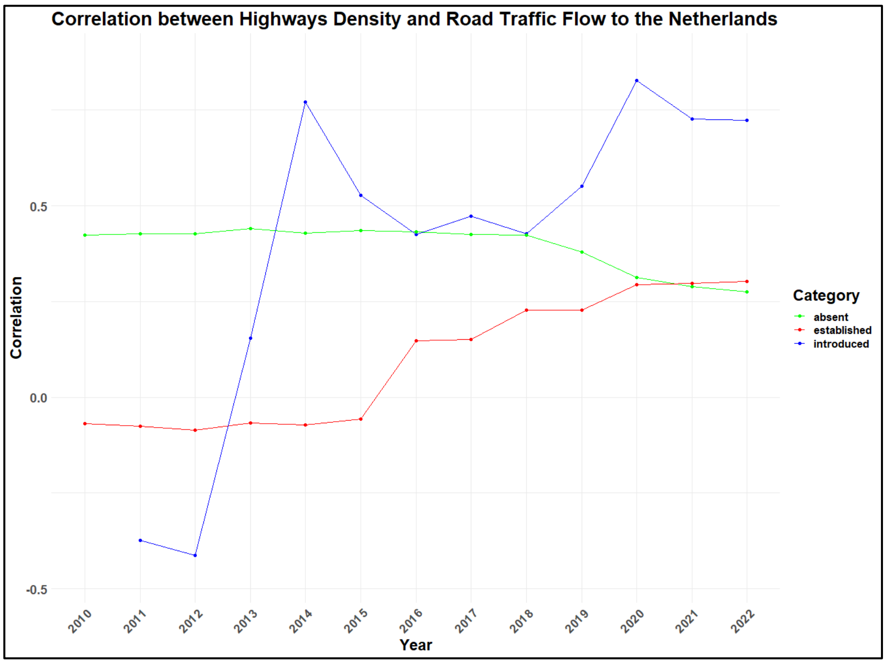

Figure 27: Correlation coefficients of the relationship between highway densities of regions from each establishment category and holiday road traffic flow to the Netherlands over the years

Boxplots were generated for each year spanning from 2010 to 2022. Each boxplot provides a visual representation of how the total holiday road traffic flow differs among regions within each establishment category for a specific year. For every year the boxplots are shown in GIF 11. Over the years, the median values for established regions appear to remain relatively stable. Similarly, the median values for the absent category also show a consistent trend, but with more noticeable fluctuations. In contrast, the introduced category exhibits the highest level of fluctuation, particularly in recent years. Outliers are notably present, particularly in the absent regions. However, there is a gradual increase in outliers for established regions over time. Conversely, the introduced regions show minimal outliers, with exceptions observed mainly in the last few years.

As illustrated in **Figure 28**, the median value for established regions remains relatively stable throughout the entire time period, experiencing only a slight decrease in the first year it is present. Similarly, the median for absent regions displays small fluctuations, although slightly more than that of the established regions. Notably, in more recent years, the median for absent regions tends to be lower compared to earlier years. In contrast, the median for introduced regions exhibits a distinct pattern. Initially, it starts with a low median in the early years, followed by periods of increase and subsequent decrease. However, in the latter half of the time period, the median stabilizes a bit more, remaining relatively constant. Interestingly, while the median for introduced regions was lower than that of absent and established regions in the first half, it surpasses them in the latter half.

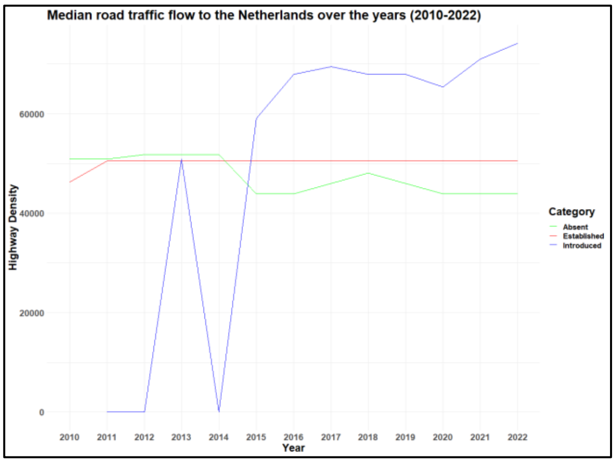

Figure 28: Median holiday road traffic flows for each establishment category over time

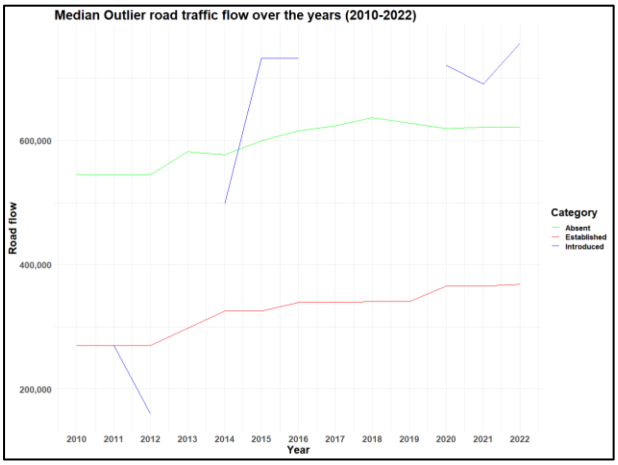

Figure 29: Median outlier holiday road traffic flows for each establishment category over time

Figure 29 shows the outlier distribution across categories. Both the absent and established categories follow a comparable pattern, where absent outliers consistently surpass those of the established category. However, the introduced category presents a different trend. The gaps represent the absence of outliers in certain years, followed by a few lower outliers in 2011 and 2012. As the years progress, the introduced outliers show higher values compared to the other categories.

Statistical tests were conducted to assess the differences in medians between the categories for each year. The Shapiro-Wilk Test for normality provided a p-value of 4.928e-07, indicating no normality in the data. Subsequently, Levene’s Test was employed to test for homogeneity in variances, resulting in a p-value of 0.007271, below the 0.05 significance level, suggesting a lack of homogeneity in variances. Given the non-normality and non-homogeneity in the data, the Friedman Test was determined suitable for the data. The Friedman Test yielded a p-value of 0.1738, which is higher than the significance level. This implies that no significant differences exist in the medians of the establishment categories across the years. To test if there indeed are no differences, a Nemenyi post-hoc Test was conducted. The detailed results of the Nemenyi post-hoc Test are presented in **Table 13**.

Table 13: Nemenyi post-hoc Test results – holiday road traffic flow

	Absent	Established
Established	0.92	-
Introduced	0.36	0.59

In addition, a Wilcoxon Test was executed to assess specific paired comparisons between consecutive years for each category. The results of the Wilcoxon Test across all combined years indicated consistently high p-values, all close to 1. These high p-values suggest a lack of evidence to reject the null hypothesis, implying that there is no significant difference in the distributions of values within each category between consecutive years.

Figure 30 presents a map showing the total holiday traffic flow from each NUTS level 3 region that each region of the selected countries receives. This is of the years from 2010 to 2018, providing an average per year. Southern Spain receives a relatively high road traffic flow, the same applies for Southern France, and the regions around Paris. Conversely, Belgium receives comparatively less road traffic flow, while the Netherlands receives some road traffic flow, albeit less than France, Spain, and Italy. France stands out with the highest share at 49%, followed by Spain at 23%, Italy at 20%, the Netherlands at 6%, and Belgium at the lowest with only 2%.

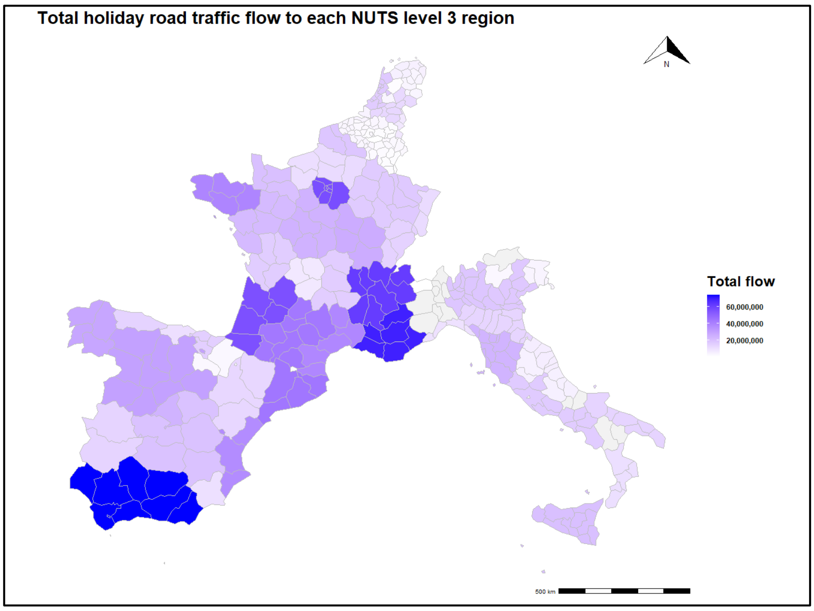

Figure 30: Total holiday road traffic flow to each NUTS level 3 regions in the selected countries – total flow received by each region

The following maps show the total holiday road traffic flow originating from each establishment category for all the regions from the selected countries, depicting the total destination road traffic flow received by each region in a specific year. GIF 12 showcases the holiday road traffic flow from the established regions to every destination NUTS level 3 region for each year. GIF 13 shows this for the introduced regions, while GIF 14 shows this for the absent regions.

Figure 31 displays a line chart depicting the fluctuations in total holiday road traffic flow from the established-, introduced-, and absent category regions over the years. Up to 2016, the total holiday road traffic flow from the absent regions remains the highest. However, after this, the road traffic flow from the established regions, which had been steadily increasing, surpasses the others, particularly in recent years, showing a notable difference. In contrast, the introduced regions consistently exhibit the lowest outgoing holiday traffic flow.

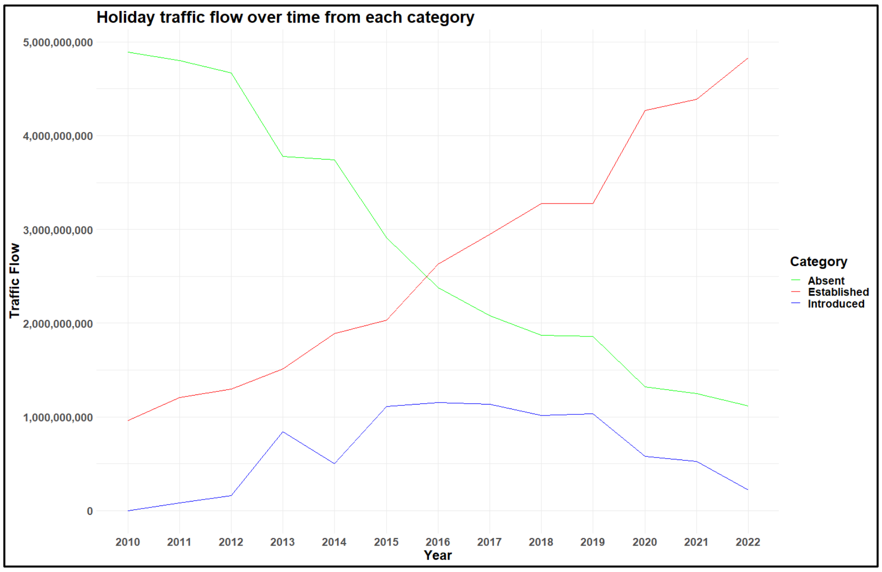

Figure 31: Total holiday road traffic flow from each category in between the regions of the selected countries

Figure 32 shows the variations in holiday road traffic flow percentages from each category with the destination the Netherlands. It represents the proportion of holiday road traffic flow directed towards the Netherlands from each category across different years. Since 2018, there has been a significant increase in road traffic flow from the introduced regions to the Netherlands, peaking at 1.78% in 2022. Meanwhile, the absent regions show a more consistent, and more gradual, increase. In contrast, traffic flow from the established regions remains relatively low, maintaining stability around 0.33% in recent years.

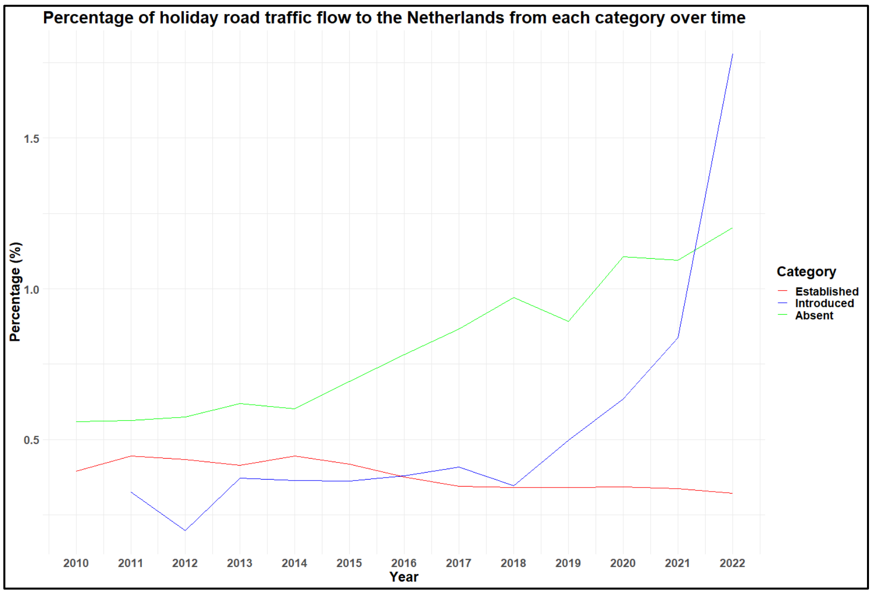

Figure 32: Percentages of holiday road traffic flow to the Netherlands from each establishment category over time

3.3. Identifying high risk areas

The aim of this third sub-research question was to determine how road networks in the Netherlands and Europe contribute to the potential introduction and distribution of *Aedes albopictus* in The Netherlands, with pinpointing possible high-risk areas. The results of the GIS analysis with the focus on distances from Asian Tiger Mosquito findings to highways, incoming holiday road traffic calculations, correlation calculations, and a prediction analysis on the potential establishment in the Netherlands, will be presented.

3.3.1. Road traffic correlation analysis

The findings of *Aedes albopictus* mosquitoes in the Netherlands between 2016 and 2022 are distributed across various postcodes. **Figure 33** shows the locations of mosquito findings per postcode location. In total there are 149 findings in twenty-nine unique postcode areas. The majority of findings are concentrated in the South of the Netherlands, particularly in Limburg, followed by Noord-Brabant and some in Zuid-Holland. However, notable concentrations of findings are also observed in certain postcodes in Overijssel and Gelderland. Other provinces that have findings are Noord-Holland, Utrecht, and Drenthe.

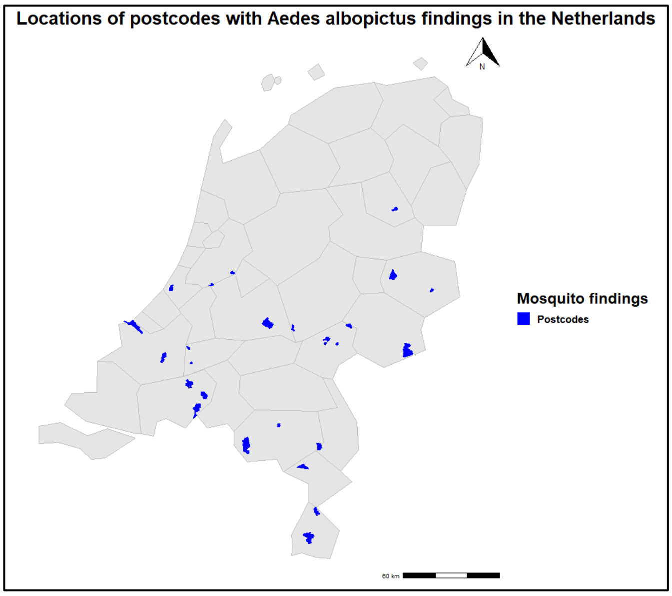

Figure 33: Locations of postcodes with Aedes albopictus findings in the Netherlands

Locations in Limburg, such as Sittard and Valkenburg, have the highest number of mosquito findings as shown in **Figure 34**. They have a total of respectively 35 and 27 findings. Similarly, in the provinces of Overijssel and Gelderland, Nijverdal and Aalten emerge as hotspots for mosquito findings. In contrast, other locations exhibit lower numbers of findings, with most postcodes having just one or a few findings. **Figure 35** shows that the more recent years generally exhibit higher average numbers compared to the earlier years. In particular, the year 2020 recorded the highest number of findings, with thirty-five occurrences.

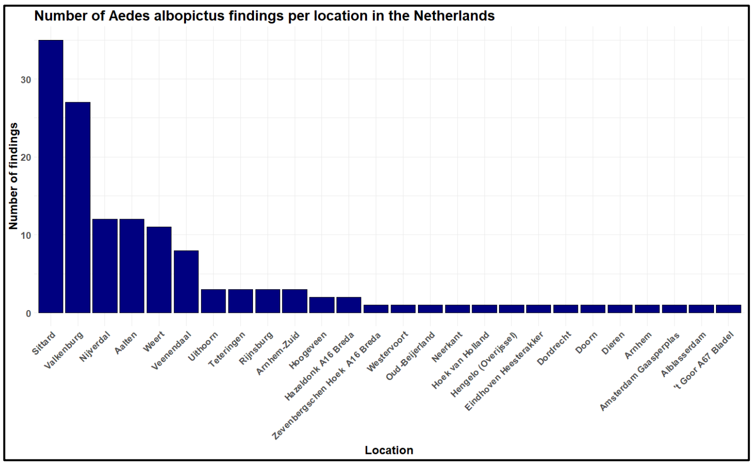

Figure 34: Number of Aedes albopictus findings per location in the Netherlands

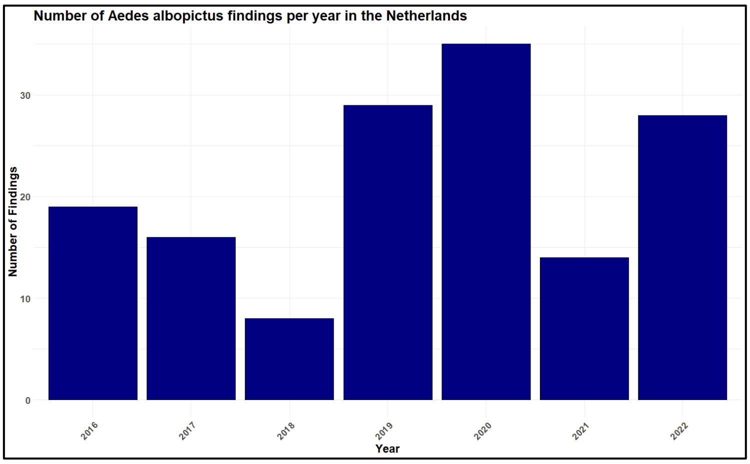

Figure 35: Number of Aedes albopictus findings per year in the Netherlands

From **Figure 36** can be concluded that eleven distinct postcode areas intersect with highways, indicating that highways are present within their area. These postcode areas correspond to fifty findings, out of the total 149 findings overall throughout the years, accounting for approximately 33.56% of all findings. Notably, from the postcodes with a high number of findings, the postcodes of the locations of Valkenburg and Weert intersect with highways.

For postcode areas that do not directly intersect with highways, the distances to highways remain relatively short. Specifically, eight postcodes are within one kilometre of a highway, while only 4 postcodes are situated at a distance of 5 kilometres or more from a highway. On average, the distance from a highway for all postcodes with findings is 1.57 kilometres. With the lowest value being zero kilometres from a highway and the highest value being 10.4 kilometres from a highway (Aalten).

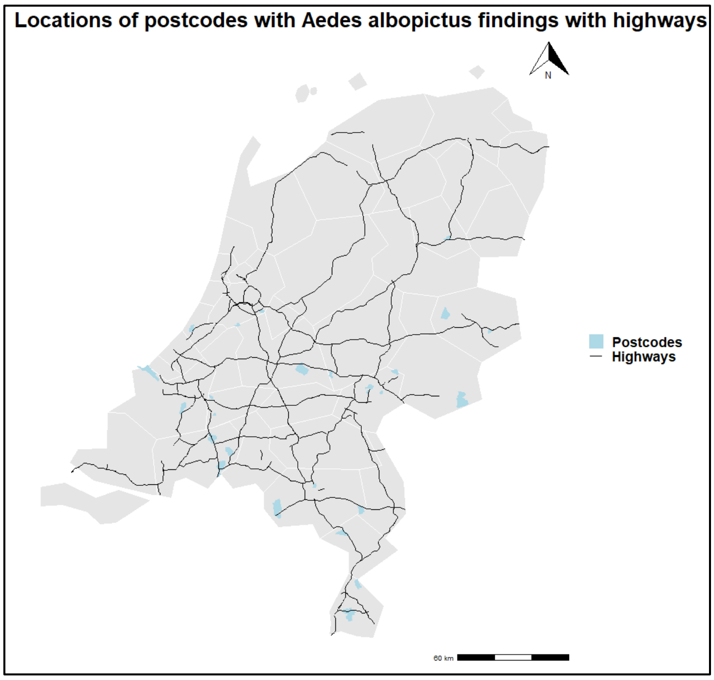

Figure 36: Locations of postcodes with Aedes albopictus findings overlayed with highways in the Netherlands

Now looking at the incoming holiday road traffic flows into the Netherlands, **Figure 37** illustrates for the established category a steady increase over time, initially starting from a low point in the earlier years. Conversely, the absent category shows a different pattern, starting high and gradually declining. Interestingly, from 2020 onwards, the established category surpasses the absent category, becoming the category with the highest total holiday road traffic flow to the Netherlands. Meanwhile, the introduced category begins with a low total traffic flow, showing slight fluctuations over the years but consistently remaining the lowest among the categories.

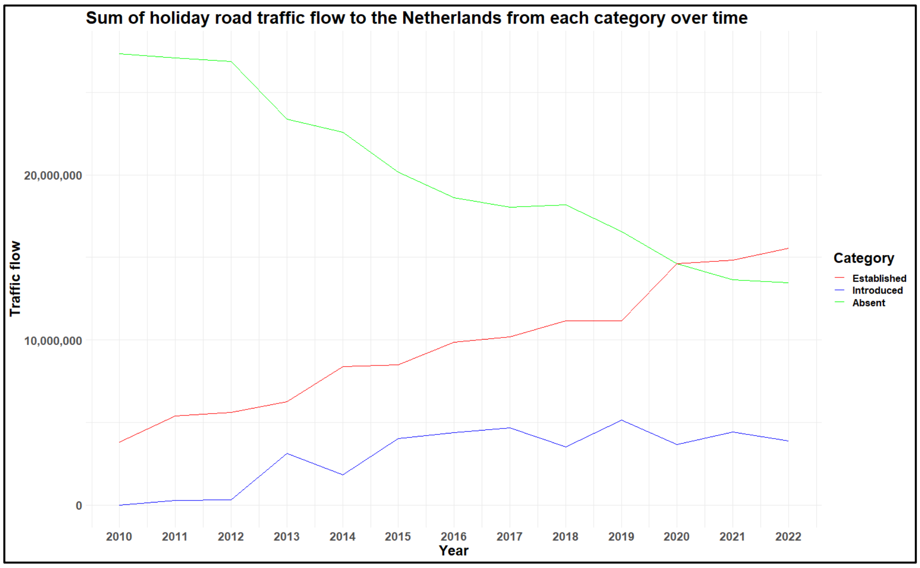

Figure 37: Sum of holiday road traffic flow to the Netherlands over time from each category

Figure 38 and **Figure 39** show the total holiday road traffic flow from each establishment category to the regions of the Netherlands, focusing solely on the year 2022. Across all categories, the provinces of Noord-Holland and Zuid-Holland consistently receive the highest volumes of road traffic, while Flevoland and Drenthe receive the least. Notably, Noord-Holland consistently ranks highest in terms of incoming road traffic flow across all categories. While the introduced category exhibit similar distribution patterns, the established category shows some deviations in the order of the amount received per province, albeit only slightly different.

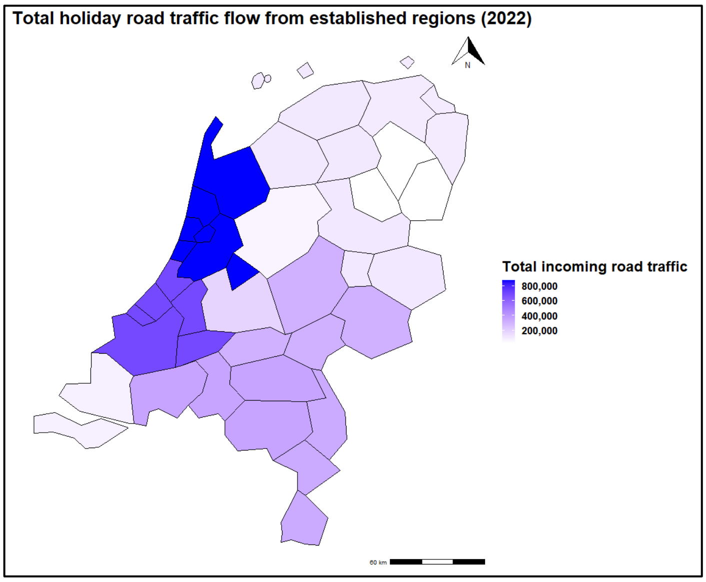

Figure 38: Total incoming holiday road traffic flow from the established category to the Netherlands in the year 2022

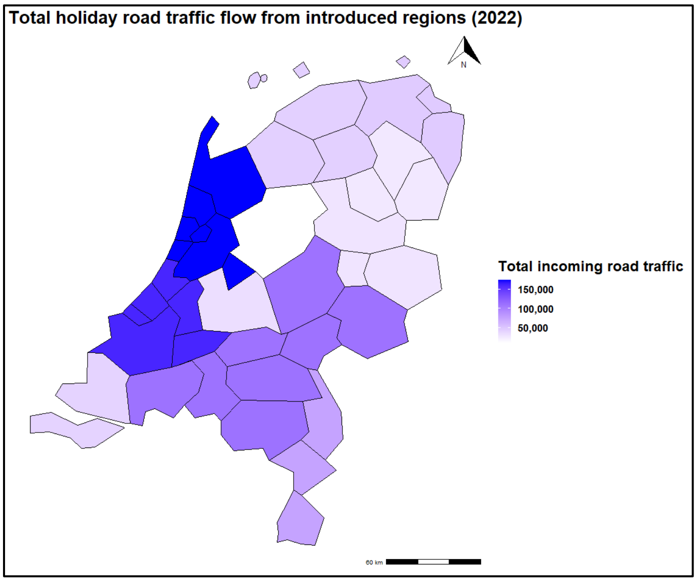

Figure 39: Total incoming holiday road traffic flow from the introduced category to the Netherlands in the year 2022

Population density was used as a factor in the correlational calculations between other variables. As can be seen in **Figure 40**, the provinces of Noord-Holland and Zuid-Holland exhibit the highest population densities, whereas regions in Zeeland and the Northern parts of the Netherlands display comparatively lower density levels.

The correlation coefficients between the total incoming holiday road traffic flow into the Netherlands for each region and the population density of each region are as followed for each category: established (0.72), introduced (0.69), and absent (0.67). These coefficients suggest a moderately positive correlation between holiday road traffic flow and population density for all categories, with the strongest correlation observed in the established category. The correlation coefficient between the distance in highways in kilometres and the population density gives a correlation coefficient of -0.13. This suggests a weak negative relationship between the distance to highways and population density.

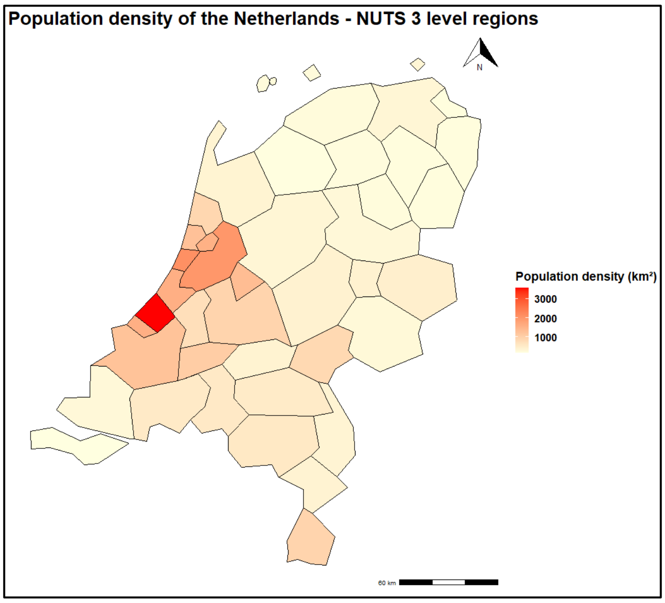

Figure 40: Population density of the Netherlands on NUTS 3 level regions scale

3.3.2. Potential movement analysis

Figure 41 and **Figure 42** are depicting the projected *Aedes albopictus* establishment years for each region in the Netherlands, based on the distribution averages of the two different methods of the first sub-research question: the closest neighbour method and the 'next-next-neighbour' method. The maps are overlaid with highways, creating an insight into the possible route the mosquito could use.

According to the closest neighbour method, the prediction suggests a more detailed time pattern, indicating that regions in the Southern part of the Netherlands are likely to become established by 2025, followed by a gradual expansion towards the Northern regions, reaching full establishment of the country by 2028. On the other hand, the 'next-next-neighbour' method predicts a quicker establishment timeline, with all regions in the Netherlands anticipated to be established as early as 2024. There is a clear difference between the predictions in years of the two methods.

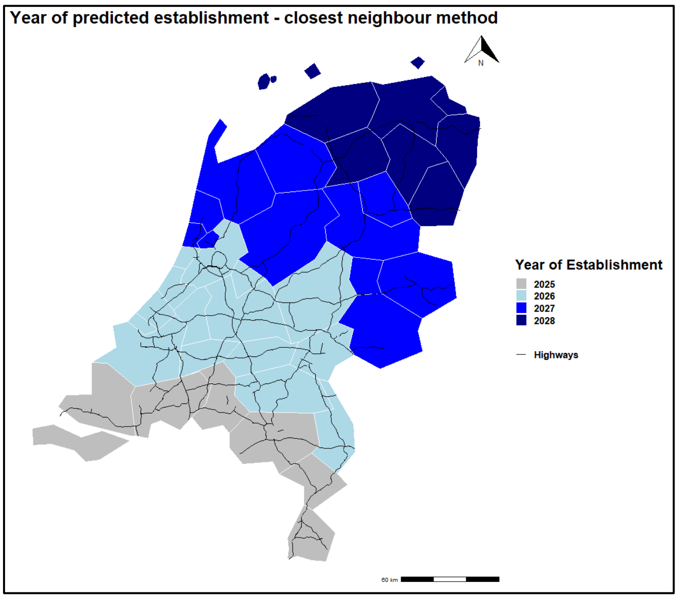

Figure 41: Year of predicted Aedes albopictus establishment for regions in the Netherlands – closest neighbour method

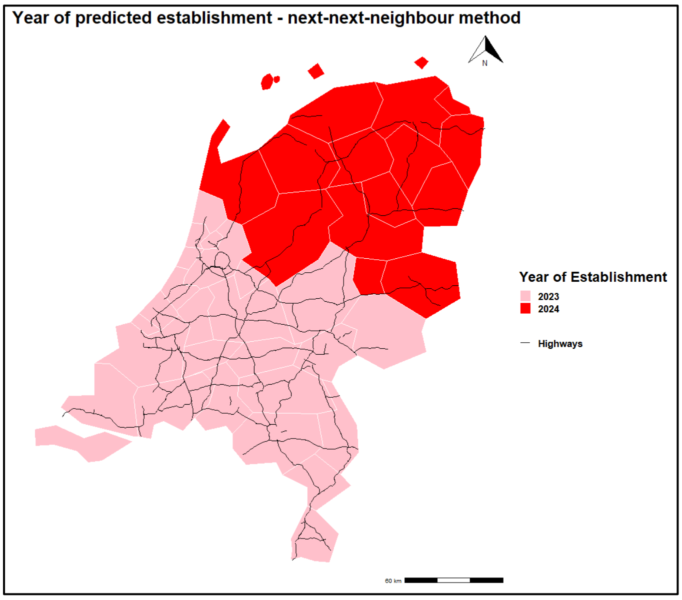

Figure 42: Year of predicted Aedes albopictus establishment for regions in the Netherlands – 'next-next-neighbour' method

4. Discussion

The research focussed on determining the potential risks zones for the introduction and distribution of *Aedes albopictus* in the Netherlands via the road traffic transport pathway. However, there are still uncertainties about the results, considering the methods and assumptions used in this research. First this will be discussed for each of the sub-research questions. After that, some constraints about the used data in this research will be addressed.

4.1. Range expansion

The first sub-research question focussed on how the range expansion of *Aedes albopictus* in Europe evolved over time, with considering what key factors can explain these changes.

The investigation into the temporal range expansion of *Aedes albopictus* in Europe from 2010 to 2022 has led to the computation of two overall average distances using distinct approaches for the closest neighbour method, resulting in approximate values of 75.88 km and 84.15 km per year. The approach focusing solely on established regions is deemed less susceptible to biases of misclassification, making the presumed actual result more likely to be around 84.15 km over the specified period. The Local Moran's I approach calculates average distances, allowing for the adjustment of parameters like the significance threshold and quadrant classifications. This flexibility can lead to varied outcomes. In addition, the determination of the four closest distances might influence results, with higher or lower closest distances possibly providing different results.

The found averages can be compared to known flight ranges - and range expansion studies of *Aedes albopictus,* which have been extensively studied. Various factors have been identified influencing the flight patterns of *Aedes albopictus* mosquitoes, including oviposition site availability, climate conditions, terrain, vegetation, housing characteristics, and blood source (Honório et al., 2003). Despite considerable research, it is consistently found that *Aedes albopictus* has limited dispersal capability over long distances. Females demonstrate autonomous dispersal over short distances, with observations in Missouri, USA, showing dispersal of up to 525 m for females and 225 m for males (Niebylski & Craig, 1994). Compared to other Aedes species, *Aedes albopictus* has weaker flight capability, with average maximum flight distances reported as 676 m compared to 333 m for Aedes aegypti (Verdonschot & Besse-Lototskaya, 2014). Studies in dengue-endemic areas have shown that *Aedes albopictus* and Aedes aegypti can cover distances of at least 800 m over a six-day period (Honório et al., 2003). In Rome, Italy, *Aedes albopictus* mosquitoes were found to have a mean daily dispersal of 119 (Marini et al., 2010), and 90% of a studied *Aedes albopictus* population had a flight range exceeding 200 m in another study (Marini et al., 2019). When considering the last study mentioned the yearly range could hypothetically be estimated at 73 km, which is close to the range expansion found of 75.88 km.

Interestingly, while individual dispersal distances may seem limited and difficult to convert to yearly estimates, the collective spread of *Aedes albopictus* in Europe is notable. The estimated rate of spread has been reported as approximately 100 km per year, accelerating to up around 150 km per year over the past five years (Kraemer et al., 2019). The average distance overall per year found in this analysis corresponds to 75.88 km per year for the Local Moran's I approach and 84.15 km per year for the established regions approach. The research of Kraemer was based on estimates of speed of spread in km per year, which are derived from thin spline regression on mosquito observations since their earliest detection in both the USA and Europe from 1990 to 2017. The variation in range expansion between Kraemer's results and this research's results could be attributed to differences in the time frames of mosquito observation data and the utilization of different methodologies.

Furthermore, the utilization of the 'next-next-neighbour' method found average distances of respectively 335.46 km for the Local Moran's I approach and 353.76 km per year for the established regions approach, which is higher than the average found by Kraemer. This difference may be attributed to the addition of an extra neighbour to the existing four closest neighbours. The inclusion of this extra neighbour introduces regions that are more distant from the original region where the average distance is calculated from, potentially causing bias. For instance, this approach might involve regions that are not directly connected or are actual neighbours, or actually are in the area. Consequently, such an inclusion in the calculations could lead to misleading outcomes in the average distance results, resulting in higher average distances and increasing trends, rather than the decreasing trend observed with the four closest regions approach.

In the determination of the range expansion, the analysis included NUTS level 3 regions that were (on) islands, although the primary emphasis was on evaluating dispersal through road traffic transport in mainland Europe. Initially, overseas territories and overseas islands were excluded from the dataset. However, islands that are part of mainland Europe, for example Corsica, remained in the analysis and therefore had an influence on the results, particularly evident in the average distances derived from the 'next-next-neighbour' method for the earlier years in France. The inclusion of islands on NUTS level 3 region scale could therefore have a certain effect on the resulting average distance.

In the case of the Netherlands, the absence of established regions during the specified time period resulted in its exclusion as a hotspot region in the Local Moran's I analysis. It predominantly features introduced and absent regions, leading to the non-identification of hotspots in the analysis. This absence had a notable impact on the distance analysis, which focused exclusively on hotspot or established regions, which were not located in the Netherlands. Consequently, the Netherlands is not considered in the results of the distance analysis. Nonetheless, the insights gained from the distance analysis still could offer valuable predictions regarding the potential distribution of *Aedes albopictus* to the Netherlands.

4.2. Holiday road traffic flows on highways

The second sub-research question focussed on to what extent holiday road traffic flow on highways connecting the South of Europe and the Netherlands contribute to risk zones for the introduction and distribution of *Aedes albopictus* in the Netherlands.

With the selection of the countries, certain countries, such as Switzerland and Germany, were excluded due to data limitations and choices made, even though the holiday road traffic flow from Germany to the Netherlands could possibly play a significant role in the potential spread of the mosquito via highways to the Netherlands. Additionally, all regions within the chosen countries were included in the analysis. For instance, in Spain, the Northeast regions consistently remained absent throughout the entire period. The decision to include or exclude certain countries and regions could give potential bias in comparing highway densities with mosquito establishment categories. Therefore, it is important to note that a different selection of countries or the inclusion/exclusion of specific regions could give different results.

The first part of the route analysis investigated the prevalence of highways across the selected regions, in regard to the establishment categories. The results indicated notable differences among the categories in terms of highway presence. Specifically, introduced regions consistently demonstrated a high occurrence of having highways across most years, whereas the presence of highways in other categories was relatively lower, albeit still high. However, a study conducted in a region in Guatemala found no significant association between the distance to highways and the abundance of Aedes aegypti larvae and pupae. The proximity of highways did not have a noticeable impact on the number of mosquito larvae and pupae, suggesting that other factors than the proximity of highways may have a greater influence on the mosquito abundance (Madewell et al., 2019). On the other hand, a study on the Bermuda Islands, regarding Aedes aegypti and *Aedes albopictus*, did found a significant relationship between mosquito density and distance to roads (Kaplan et al., 2010). In the context of this study, it is important to acknowledge that while highway presence may play a role, other factors besides proximity to highways may also influence the prevalence of *Aedes albopictus*.

Highway density may be one of those other factors. The relationship between the highway's densities of each region with regards to the establishment categories was determined in this research. A closer examination of the medians of the resulting boxplots and spatial distribution reveal that outliers in the introduced and absent categories were primarily associated with regions in Belgium and the Netherlands. These regions were either absent or introduced during the study period and have higher highway densities compared to other regions within the selected countries. On the other hand, the established regions cover larger areas, with their overall highway density being generally lower. While there is not much known about the effect of highway density and mosquito prevalence, some studies suggest that highways act as a barrier for mosquito distribution and prevalence. For instance, a study conducted in Trinidad and Tobago, observed that a large urban highway has a notable effect on the movement of Aedes aegypti mosquitoes. The highway essentially serves as a significant obstacle to their distribution, suggesting that the mosquitoes could not cross such highways (Hemme et al., 2010). In addition, in Queensland, Australia, a study found that Aedes aegypti mosquitoes were less likely to cross a major highway compared to smaller roads, suggesting that busy roads could restrict their dispersal (Russell et al., 2005). In the context of this study, the observed high highway density in Belgium and the Netherlands, particularly in introduced regions, may suggest that *Aedes albopictus* could face challenges in dispersing itself within the Netherlands, when highways may act as a barrier. However, considering the distribution pathway of road traffic transport (hitchhiking) via highways, this obstacle might be avoided, as higher highway density could provide more opportunities for distribution in this scenario.

The next part of the analysis focussed on the relationship between holiday road traffic flow and the establishment categories. Looking at the road traffic flow from the Netherlands, when Dutch tourists travel within Europe, car travel accounts for 54% of their transportation choices (CBS, 2021). During the returning from holiday destinations, there is a risk of the mosquito 'hitchhiking' in the Dutch tourists' cars back to the Netherlands. The provinces of Zuid-Holland and Noord-Holland receive the highest holiday road traffic flow from the selected countries. This result aligns with data from the Dutch Central Statistical Office (CBS), which highlights these provinces as major tourist destinations, primarily due to attractions in cities like Amsterdam, Rotterdam, and The Hague (CBS, 2022). The notable incoming holiday traffic flow from Belgian regions, the highest compared to the others, can be attributed to Belgium's proximity to the Netherlands, encouraging more car travel. Nearly 80% of Belgian tourists make use of the car when visiting the Netherlands, whereas the percentages are lower for France (30%), Spain (4%), and Italy (6%) (CBS, 2021). Notably, this takes into consideration only the tourists travelling to the Netherlands but not the travelling back of the Dutch tourists to the Netherlands.

A weak positive correlation between highway density and incoming holiday road traffic flow to the Netherlands was found. However, looking at each category, a strong positive correlation was observed for the introduced regions in recent years. This stronger correlation can be attributed to the fact that most of the introduced regions in the recent years were located in Belgium. In regions where highway density is high, there is a greater likelihood of high incoming holiday road traffic flow to the Netherlands*,* facilitating the potential distribution of *Aedes albopictus* via the road traffic transport pathway from these regions. Additionally, the correlation results suggests that *Aedes albopictus* may have a higher chance of entering the Netherlands from introduced regions rather than established ones, as the correlation between highway density and incoming holiday road traffic flow is stronger for introduced regions, with the correlation being weak for established regions.

Another study highlights that dengue fever cases in Italy, Spain, and France are predominantly concentrated in tourist hotspots, particularly along the Mediterranean coast, with additional clusters observed in major urban hubs like Paris and Madrid. These regions attract a significant influx of incoming and returning travellers who may have visited dengue-endemic areas (Brem et al., 2024). This underscores that holiday road traffic flows could correlate with the spread and distribution of mosquito vectors, primarily from established regions like in France and Spain. Within the scope of this research, the potential spread from these established regions to the Netherlands may also imply a similar relationship. However, the correlation for established regions to the Netherlands was identified as weak, but with a strong correlation for introduced regions.

The boxplots illustrating holiday road traffic flow in relation to the establishment categories suggest an influence of introduced regions in Belgium, particularly in recent years. This influence results in a higher overall median for the introduced category compared to the other two categories in recent years particularly. The increased median implies that holiday road traffic flow from Belgium to the Netherlands is higher than from other regions, which aligns with the spatial proximity of Belgium to the Netherlands. Moreover, considering the strong correlation observed for introduced regions in recent years, there is an increased likelihood of distribution and spread of *Aedes albopictus* to the Netherlands via Belgium with the road traffic transport pathway.

Research conducted in Belgium (Deblauwe et al., 2022) provides further evidence supporting this statement. It suggests that the increasing population of *Aedes albopictus* in Northern France and Germany is likely caused by mosquitoes found at parking spots in Belgium, with travel distances of approximately 2 to 2.5 hours from these new introduction populations. Parking lots in Belgium, often the first point of entry for vehicles arriving from colonized areas in Northern France and Germany, have detected these introduced mosquitoes. Recent detections at parking lots near the Northern Belgian-Dutch border, and subsequent introductions into the Netherlands, highlight the potential for *Aedes albopictus* introduction across Belgium. Furthermore, it predicts a rise in short-distance introductions in the coming years, indicating an ongoing risk of mosquito spread via this pathway on the way to the Netherlands.

The overall holiday road traffic flow across regions in the selected countries reveals that the Netherlands represents only 6% of the total destinations. Yet, there is a notable upward trend in the total holiday road traffic flow from established regions over the years, suggesting a potential increase in *Aedes albopictus* distribution across Europe via these regions. While the proportion of road traffic destined for the Netherlands remains relatively low, recent years have seen a significant rise in the proportion of traffic from introduced regions headed to the Netherlands. This is also likely linked to the increase in introduced regions, particularly in Belgium in the recent years. This trend raises concerns for the future, especially as regions in Belgium become established. With already high traffic flow from these regions, there is an increased risk of mosquito distribution and introduction in the Netherlands. However, considering the high highway density in the Netherlands, *Aedes albopictus* may face challenges in establishing itself, as suggested by research indicating a decrease in flying insect abundance in locations with increasing road traffic (Martin et al., 2018).

4.3. Identifying high-risk areas

The third sub-research question focussed on determining how road networks in the Netherlands and Europe contribute to the potential introduction and distribution of *Aedes albopictus* in The Netherlands, with pinpointing possible high-risk areas.

In the calculation of distances from highways, the total distance in kilometres is determined based on the closest distance from the border of a postcode area with findings, rather than the centroid of the postcode area. This approach acknowledges that the actual findings may occur in the part of the postcode area farthest from the highway. However, due to the aggregation of postcodes without the last two letters, there is a potential bias in the results. For instance, while the finding may be far from the highway, considering the entire postcode area might have a closer proximity to a highway. Consequently, this could affect both the number of unique postcode findings intersecting with highways and the average distance in kilometres from all postcodes with findings. It is essential to note that the analysis solely focuses on highways, yet other roads, such as provincial roads, could also influence the distribution of *Aedes albopictus* in the Netherlands. A study in Trinidad supports this statement, indicating that areas near minor motorways serving minor towns and villages, accounting for 30% of the population, experienced a higher prevalence of dengue cases, a disease transmitted by Aedes mosquitoes, compared to regions adjacent to major motorways where 60% of the population resides (Mahabir et al., 2012).

Although the introduced regions exhibit the highest proportion of incoming holiday road traffic flow to the Netherlands compared to other categories, the total sum of incoming road traffic flow in the Netherlands remains the lowest for this category. Nevertheless, the regions in the Netherlands that receive the most road traffic are relatively consistent across all categories. This aligns with the results of the second sub-research question, which highlighted Zuid-Holland and Noord-Holland as the provinces receiving the highest holiday road traffic. Overall, there is minimal disparity in the rankings of total road traffic received among regions, both across all categories together and within each specific category. This could imply that the likelihood of *Aedes albopictus* arriving in Zuid-Holland and Noord-Holland is higher compared to provinces with lower incoming holiday road traffic flow. This is particularly significant given the increasing total amount of road traffic flow of the established category, which also add to the growing potential of *Aedes albopictus* 'hitchhiking' from established regions to those provinces.

A correlation analysis was conducted between the total incoming holiday road traffic flow for each category and region, and the population density of each region. The choice of including population density as a factor was chosen based on a scientific review, which highlighted the relationship between urbanization and the distribution and density of Aedes mosquitoes. Notably, the review suggested that human population density exceeding 1.000 people per square kilometre was linked with elevated levels of arboviral diseases (Kolimenakis et al., 2021). In the Netherlands regions with a population density higher than 1.000 people per square kilometres are only found in the provinces of Noord-Holland and Zuid-Holland. Those provinces also receive the most incoming holiday road traffic flow. The relationship found for each category between the incoming road traffic and the population density are of moderately positive strengths. This indicates that regions with higher population densities, such as those found in the provinces of Noord-Holland and Zuid-Holland, are more likely to experience increased incoming holiday road traffic flow, potentially enhancing the risk of mosquito introductions and spread in these areas.

There was no relationship found between the distance of the postcodes with findings to highways and the population density. Although population density was calculated based on NUTS level 3 regions and distance to highways was determined at the postcode level, utilizing population density at the postcode level could potentially give another result.

The predictions regarding the potential establishment of regions in the Netherlands is based on averages derived from the first sub-research question, which have their own constraints and limitations, as previously discussed. According to the 'next-next-neighbour' method, the Netherlands as a whole is projected to become established by 2024, while the closest neighbour method suggests 2028. A comparison with the current distribution maps from the ECDC (as of October 2023) reveals that almost all regions in the Netherlands have been introduced, except for Zeeland, Friesland, and Groningen, which remain absent. Looking at this, the 'next-next-neighbour' method provided an inaccurate prediction, while the closest-neighbour method prediction remains a possibility. However, other factors may also influence the potential establishment of *Aedes albopictus* in the Netherlands, potentially also affecting the averages utilized in this prediction.

4.4. Data constraints

NUTS data

The NUTS dataset posed challenges in interpreting the results due to variations in the size and level of detail of the NUTS level 3 regions across different countries. For instance, Germany contains of numerous small regions, whereas Spain encompasses larger ones. This variation in region sizes, when combined with for instance the mosquito data, resulted in differing levels of detail, potentially affecting the interpretation of the mosquito spread patterns. Although the dataset cannot be altered, it is important to acknowledge that differences in regional area sizes could influence the interpreting of the results.

Mosquito data

There were some specific misclassifications in the data, such as the 'Kop van Noord-Holland' region in the Netherlands, which was found to be misclassified in a certain year, and rectified. The possibility of unidentified errors remains a possibility in the dataset. Therefore, a comprehensive review and validation process are essential to ensure the dataset's accuracy and usability. Moreover, refining rules for data surveillance or research classifications, such as distinguishing between 'established' and 'introduced' classified regions, is crucial for enhancing data quality and obtaining more reliable results. Additionally, while comparing distribution maps generated in this research with those available on the ECDC website, it was observed that the research maps provide a more detailed overview due to their granular nature with the use of NUTS level 3 regions. Despite this difference in detail, the overall results remain consistent across both sets of maps.

Road data

The road dataset used in this research was last updated in 2017, placing it in the middle of the research period (2010-2022). However, potential issues could arise from this, as the highway data only reflects conditions as of 2017. Changes such as the construction of new highways or the discontinuation of existing ones may introduce inaccuracies in the calculated highway parameters. Ideally, having annual highway data throughout the study period would offer a more accurate representation, but such detailed data is unavailable or yet to be found. Moreover, the process of filtering highways based on specific column values in the road dataset could lead to the possibility of selecting highways incorrectly. However, when comparing the reported total highway length for the countries in the dataset with the updated real-life highway lengths, no considerable significant deviations were observed. Differences from the dataset with the real-life situation being 81 km for Belgium, 366 km for France, 147 km for Italy, 13 km for the Netherlands, and 461 km for Spain (Wegenwiki, n.d.). This suggests a reasonably accurate reflection of highway coverage in the selected countries based on the used road dataset. An alternative data source, like OpenStreetMap, could provide more up-to-date highway information. However, due to time - and technical constraints, accessing this data for various national levels was not feasible in this research.

Number of Trips data

The ‘Number of Trips’ dataset spanned from 2010 to 2018, representing the average total ‘Number of Trips’ (holiday road traffic flow) per year. This dataset was combined and compared with the mosquito establishment data ranging from 2010 to 2022. While there exists a time gap between these datasets, the ‘Number of Trips’ dataset offered an estimation of traffic flow from NUTS level 2 regions, facilitating as use in this research. It is important to acknowledge that the actual holiday road traffic flow may differ, and the averages presented could be influenced by various factors. For instance, the dataset's construction involved a complex methodology, and true estimates of holiday road traffic flow on such a large scale are challenging to obtain. Additionally, external factors like the impact of major events such as the COVID-19 pandemic were not accounted for in the analysis, which potentially could lead to biases in the results. Thus, while the dataset provides valuable insights, it is essential to interpret the results with caution and recognize the potential limitations.

The ‘Number of Trips’ dataset was filtered to include only trips with the Netherlands as the destination. However, this filtering process resulted in the exclusion of numerous regions within the selected countries. Furthermore, some regions had zero traffic flow directed towards the Netherlands, further reducing the number of included regions. Consequently, only a limited number of regions from each country could be considered in the analysis, potentially introducing biases into the results. Moreover, the dataset's coverage was not optimal, with gaps in the inclusion of regions on route to the Netherlands. These missing data regions could have influenced the outcomes, as the traffic flow between regions with no recorded data remains unknown. Also, the dataset includes only one-way trips from regions to another region, without accounting for return trips.

Population density data

The population density data used in this research was available at the NUTS level 3 scale for regions in the Netherlands, whereas the postcodes dataset and the NVWA dataset were available at postcode level. This difference in detail levels could introduce bias, as the *Aedes albopictus* findings were aggregated to the population density level, losing information about the specific postcode where the mosquito was found. Using population density data at the same level of detail as the other datasets would possibly yield more accurate results. Additionally, since the population density data was only available for the year 2022, the analysis could only be conducted for that particular year.

5. Recommendations

Based on the insights from the results and discussion, some recommendations can be made. These recommendations are structured according to each research question and can serve as a basis for formulations of future research. At last, some general recommendations are given.

5.1. Range expansion

The range expansion of *Aedes albopictus* was calculated for Europe on continental scale. However, it may also be interesting to explore the range expansion analysis at national levels, as illustrated by the specific example of France. The analysis conducted in France provided more detailed results on national scale, offering insights that could contribute to more targeted and detailed surveillance efforts on national – and possibly even on regional level. Thus, it is recommended to extend similar analyses to national scales for other countries, providing a more comprehensive understanding of the dynamics of *Aedes albopictus* range expansion in other specific countries.

For future research about the possible range expansion, it is advisable to conduct, for instance, a sensitivity analysis to determine the optimal number of closest neighbours or next-next-neighbours. Alternatively, exploring alternative methods, such as calculating an average between the number of closest neighbours within a certain distance threshold, might provide better insights into determining the right number of (closest) neighbours to achieve more accurate average distance results.

Island regions were included in the range expansion analysis. Accessibility varies among islands, with some accessible by car, often through ferry routes or tunnels, while other island regions lack this kind of car-connectivity to the mainland. As a recommendation, only including islands accessible by bridge or tunnels (those with road connections), could enhance the precision of the average distances results in regard of the mosquito movement over land with ground vehicle transportation.

5.2. Holiday road traffic flows on highways

Future studies should explore different country selections and the inclusion/exclusion of specific regions to better understand the impact on holiday road traffic flows and *Aedes albopictus* distribution and introduction to the Netherlands. Utilizing OpenStreetMap data, when feasible, would provide more accurate highway information. Furthermore, research should consider external factors like the COVID-19 pandemic's influence on holiday road traffic flow and enhance data quality by incorporating two-way trip data by considering return trips.

Understanding the relationship between highway density and the establishment categories, along with its impact on *Aedes albopictus* distribution, is important for a comprehensive risk assessment within the scope of the road traffic transportation pathway. Better insights on how highways may act as barriers or facilitators for mosquito dispersal via the road traffic transport pathway is needed. Furthermore, additional research is needed to understand the varying correlation strengths between established and introduced regions regarding *Aedes albopictus* spread via holiday road traffic flows to the Netherlands. Moreover, exploring the potential impact of increasing holiday road traffic flow from introduced regions, especially in Belgium, and its relationship with high highway density in the Netherlands regarding the distribution of *Aedes albopictus* demands further investigation.

5.3. Identifying high-risk areas

Further research could enhance the distance from postcodes with findings to roads analysis by incorporating provincial or other roads beside highways. Additionally, refining the aggregation of the postcode areas based on the four numbers and two letters could generate more accurate results. Utilizing the aggregated, more detailed level of postcodes in combination with population density could also provide more precise outcomes.

Other social-economic factors beyond population density may also contribute to the higher levels of incoming holiday road traffic flow observed in provinces like Noord-Holland and Zuid-Holland. For instance, residents in these provinces may have more money, enabling them to travel more frequently, particularly to regions in the South of Europe where *Aedes albopictus* already is established. This increased travel activity raises the risk of the mosquito 'hitchhiking' back to the Netherlands. Furthermore, future research could explore those socio-economic factors at postcode scale, such as low-income areas, and their proximity to the finding's areas and highways. Investigating these and other potential influences on holiday road traffic could provide valuable insights into the potential introduction and distribution of *Aedes albopictus* in various spatial scales.

5.4. Overall recommendations

Future research could also delve into examining transportation hubs situated along highways, which encompass petrol stations, large parking areas, rest stops, and similar facilities. These locations have been identified as potential hitchhiking hotspots for *Aedes albopictus* (Eritja et al., 2017). There exists a possibility for the mosquito to hitchhike in vehicles traveling back to the Netherlands. Therefore, it is recommended for future research to consider these transportation hubs when identifying potential risk zones for the introduction and distribution of *Aedes albopictus* on the way to the Netherlands.

Moreover, future studies could employ agent-based modelling incorporating the parameters found in this study to simulate the distribution and introduction of *Aedes albopictus* in the Netherlands via holiday road traffic flows and highways.

Lastly, to ensure the overall quality and accuracy of future research, it is necessary to establish more precise rules for data surveillance and the classifications of mosquito establishment classes. Additionally, increased collaboration across countries to enhance data sharing could provide more comprehensive information about *Aedes albopictus* to organizations like the ECDC. This collaborative effort will help ensure that the ECDC's data is regularly updated with accurate and reliable information on *Aedes albopictus* distribution and establishment.

6. Conclusion

With the findings of this study several conclusions can be drawn, these are organized according to each sub-research question. Finally, a general conclusion is given based on the main research question of the research.

6.1. Range expansion

From 2010 to 2022, Europe has experienced an increase in *Aedes albopictus* distributions. Mosquito establishment classes and Local Moran's I analysis identified hotspot - and established regions, signifying this increase of establishment of the mosquito. The annual average distances found for the closest distance method, as determined by the Local Moran's I approach, was approximately 75.88 km, whereas using the established regions approach yielded a value of 84.15 km. The 'next-next-neighbour' method found considerably higher results with 335.46 km for the Local Moran's I approach and 353.76 km for the established regions approach. These differences between methods emphasize the importance of methodology in interpreting patterns of *Aedes albopictus* range expansion over time.

Consistent with existing literature, evidence suggests *Aedes albopictus'* adaptability to capitalize on human-modified environments. Considering the anticipated impacts of climate change, ongoing monitoring of these mosquitoes is crucial to further determine the influencing factors on the range expansion of *Aedes albopictus* in Europe. Given these insights, it is advisable to conduct similar analyses at national scales. Such granularity can provide perspectives essential for developing targeted strategies and interventions to manage *Aedes albopictus* across continental and national scales.

6.2. Holiday road traffic flow on highways

Regions from the selected countries classified as introduced consistently exhibited high highway presence over the timeframe of this research. Considerable high holiday road traffic flows to the Netherlands were observed from introduced regions, with regions in Belgium likely contributing to this due to its proximity to the Netherlands. The combination of high holiday road traffic flow to the Netherlands - and extensive highway networks in Belgium could suggest a potential for the distribution and introduction of *Aedes albopictus* to the Netherlands via Belgium.

The strong relationship observed between holiday road traffic flow from introduced regions, with a correlation coefficient of 0.82, underscores this increased risk of *Aedes albopictus* introduction into the Netherlands. This could indicate that regions with considerable holiday road traffic flow to the Netherlands, such as Belgium, may serve as major contributors for the spread of *Aedes albopictus* to the Netherlands via the road traffic transport pathway.

Moreover, when the now classified introduced regions in Belgium might become established, the risk of *Aedes albopictus* distribution into the Netherlands is expected to increase. When this happens a growth in holiday road traffic flow from established regions to regions in the Netherlands will be expected. This will potentially add to the mosquito's increase in the use of Belgian highways as road traffic transportation dispersal pathway. The predicted rise in short-distance introductions of *Aedes albopictus* in the upcoming years from Belgium, highlights this risk even more.

6.3. Identifying high-risk areas

The proximity to highways from postcodes with *Aedes albopictus* findings in the Netherlands, as indicated by an average distance of 1.57 km, suggests a potential usage of the road traffic transport pathway in the Netherlands. However, there are substantial differences in the range of distances from highways between *Aedes albopictus* findings in the Netherlands. Hence, the influence of other roads, such as provincial roads, should not be overlooked.

Moreover, regions with higher population densities, notably within the provinces of Noord-Holland and Zuid-Holland, experience the most incoming holiday road traffic flow in the Netherlands. This potentially enhances the higher risk for *Aedes albopictus* introduction and spread within these provinces. The most incoming holiday road traffic comes from regions classified as established in the recent years. As such, the provinces of Zuid-Holland and Noord-Holland emerge as the highest risk regions for the introduction and distribution of *Aedes albopictus* via the road traffic transport pathway in the Netherlands.

The potential movement prediction projected a possibility of the Netherlands to be fully established by *Aedes albopictus* in the year 2028. However, further research is essential to provide a more accurate prediction of the potential high-risk zones within the Netherlands for the introduction and distribution of *Aedes albopictus*, and for the timeline of possible complete establishment of the Netherlands as a whole.

6.4. Overall conclusion

To answer the main research question of this research: '*What are the potential locations at risk for the introduction of Asian Tiger Mosquitoes (Aedes albopictus) in the Netherlands through ground traffic transport?*', it can be stated, in conclusion, that this research provides amongst others, insights into the expanding range of *Aedes albopictus* in Europe. It highlights the significant role of holiday road traffic flow, particularly from regions classified as introduced, in potentially introducing and distributing *Aedes albopictus* to the Netherlands. The research identified high-risk areas, notably Noord-Holland and Zuid-Holland, where increased incoming holiday road traffic flow and high population densities heighten the risk of *Aedes albopictus* potential introduction. These findings underscore the importance of targeted interventions and continued monitoring to mitigate the risk of *Aedes albopictus* establishment in the Netherlands via the road traffic transport pathways, especially from regions in Belgium.

7. References

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8. Appendix – ZIP file data

This appendix gives a table (**Table 14**) outlining the contents of the accompanying Zip file of this thesis report. It includes all the documents utilized in this thesis research.

Table 14: Overview of contents of the corresponding ZIP file of the thesis report

Document	Format	Folder structure
README file	Word	Thesis Jorrit Millenaar > README.doc
Thesis Proposal	Word & PDF	Thesis Jorrit Millenaar > Proposal > Thesis Proposal Jorrit Millenaar.doc Thesis Proposal Jorrit Millenaar.pdf
Thesis report	Word & PDF	Thesis Jorrit Millenaar > Thesis report > GRS Thesis Jorrit Millenaar (1301268).doc GRS Thesis Jorrit Millenaar (1301268).pdf
Midterm presentation	PPTX	Thesis Jorrit Millenaar > Presentations > Midterm Presentation Jorrit Millenaar.pptx
Final presentation	PPTX	Thesis Jorrit Millenaar > Presentations > Final Presentation Jorrit Millenaar.pptx
GIFs	GIF	Thesis Jorrit Millenaar > GIF > gif_name.gif
Flowcharts 1	PNG	Thesis Jorrit Millenaar > Flowcharts > Flowcharts 1 > flowchart_name.png
Flowcharts 2	PNG	Thesis Jorrit Millenaar > Flowcharts > Flowcharts 2 > flowchart_name.png
Flowcharts 3	PNG	Thesis Jorrit Millenaar > Flowcharts > Flowcharts 3 > flowchart_name.png
Input data 1	Multiple file types	Thesis Jorrit Millenaar > Thesis R > Input data 1 > Input_data.filetype
Input data 2	Multiple file types	Thesis Jorrit Millenaar > Thesis R > Input data 2 > Input_data.filetype
Input data 3	Multiple file types	Thesis Jorrit Millenaar >Thesis R > Input data 3 > Input_data.filetype
Output data 1	Multiple file types	Thesis Jorrit Millenaar > Thesis R > Output data 1 > Output_data.filetype
Output data 2	Multiple file types	Thesis Jorrit Millenaar > Thesis R > Output data 2 > Output_data.filetype
Output data 3	Multiple file types	Thesis Jorrit Millenaar > Thesis R > Output data 3 > Output_data.filetype
R History	R History Source File	Thesis Jorrit Millenaar > Thesis R > .Rhistory
R Data	R Workspace	Thesis Jorrit Millenaar > Thesis R > .RData
Research question 1 R script	R-file	Thesis Jorrit Millenaar > Thesis R > RQ1.R
Research question 2 R script	R-file	Thesis Jorrit Millenaar > Thesis R > RQ2.R
Research question 3 R script	R-file	Thesis Jorrit Millenaar > Thesis R > RQ3.R