eman ta zabal zazu

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DIPTERA CULICIDAE VECTORS OF PATHOGENS AFFECTING ANIMAL AND PUBLIC HEALTH IN THE BASQUE COUNTRY IN A CONTEXT OF GLOBAL CHANGE

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TESIS DOCTORAL

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Authorisation Pages

A mi familia y amigos

"Science and everyday life cannot and should not be separated. In so far as it goes, it is based in fact, experience and experiment."

- *Rosalind Franklin*

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Fátima

Fátima

Presentation of the thesis

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List of Abbreviations

µl - microlitre

µM - micromolar

AIC - Akaike information criterion

AIM - *Aedes* invasive mosquitoes

APC - Animal protection centre

BLAST - Basic local alignment search tool

BOLD - Barcode of life data system

bp - Base pairs

BSA - Bovine serum albumin

***ca*.** - *circa*

CDC - Centre of Diseases Control

CI - Confidence interval

CO_2 - Carbon dioxide

COI - Cytochrome c oxidase I subunit

dNTP - Deoxyribonucleotide triphosphate

DDT - Dichlorodiphenyltrichloroethane

DNA – Deoxyribonucleic acid

DOI - Digital object identifier

e.g. - *exempli gratia*

ECDC - European Centre of Diseases Control

GLM - Generalised linear model

H’ - Shannon’s diversity index

HLC - Human landing collection

IMS - Invasive mosquito species

IRR - Incidence rate ratio

ITS-2 - Internal transcriber spacer 2

L4 -Fourth stage larvae

LRT - Likehood ratio test

MBD - Mosquito-borne diseases

NBGLM - Negative binomial generalised models

Mg - Magnesium

mg - milligram

ml - mililitre

mM - milimolar

NCBI - National Centre for Biotechnology information

NGS - Next generation sequencing

OR - Odds ratio

OTUs - Operational taxonomic units

PCR - Polymerase chain reaction

PCR-RFLP - PCR Restriction fragment length polymorphism

POI - Positive ovitrap index

s - second

S - Species richness

SN - Sweep netting

sp. - species (singular)

spp. - species (plural)

TE - Tris EDTA

UV - Ultraviolet

WN - West Nile

WNV - West Nile Virus

Summary

Mosquitoes (Diptera: Culicidae) are common bloodsucking Diptera frequently found in different environments. Understanding their distribution, abundance, and ecological dynamics is essential, particularly in the context of increasing urbanization and the emergence of invasive species. This PhD thesis aimed to address this knowledge gap by conducting a comprehensive investigation into mosquito species in the Basque Country, focusing on different environments and anthropization levels. The overarching objective is threefold: first, to identify Culicidae species across natural, rural, peri-urban, and urban settings, exploring factors shaping their presence, abundance, and richness; second, to study the distribution of invasive *Aedes* spp. species in different degrees of anthropization and assess the factors that influences them; and third, to investigate the feeding patterns of mosquito species along an urban-to-wild gradient. To address this objective four studies have been carried out.

Wetlands are valuable ecosystems for many animal species, particularly migrating birds. Therefore, interactions between animal species and mosquitoes may play a critical role in pathogen transmission. In **Study I**, mosquitoes were collected from two aquatic ecosystems using different methodologies and identified using classical morphology and molecular tools. A total of 1529 males and females of 22 native mosquito species were trapped using CO2-baited Centers for Disease Control and Prevention (CDC) traps and sweep netting. Overall, the most abundant species was *Coquillettidia richiardii*, followed by *Aedes rusticus*, *Cx. pipiens* s.l. and *Culex modestus* but the species composition varied depending on the aquatic setting. Among the blood-fed female mosquitoes, 11 vertebrate host species—six mammals and five birds—were identified using DNA barcoding. The developmental sites of eight mosquito species were determined across nine microhabitats, and 11 mosquito species were caught landing on humans. The flight period varied among mosquito species, with some peaking in the spring and others in the summer.

Simultaneously, the diversity and abundance of mosquitoes were also investigated in three animal protection centres (APCs) located in rural or suburban areas. In the second study of this thesis (**Study II**), four miniature suction CDC light traps (two UV and two standard incandescent bulb traps, both types baited and non-baited with CO_2) were placed in each APC. A total of 224 mosquitoes (8 species) were collected and identified by both morphological and molecular approaches. The *Culex pipiens/Cx. torrentium* comprised 76.8% of the Culicidae. The input of CO_2 in light traps proved less effectiveness in improving the collections of mosquitoes, and UV-light traps were 2.2 times more effective than incandescent light traps. Captures of mosquitoes were significantly larger at the beginning of the summer.

Study III was conducted in the three main cities of the Basque Country to investigate the species composition, abundance, dynamic populations, larval habitats, and host preferences of mosquitoes from urban green spaces and cemeteries. CDC traps and dipping were used to collect mosquitoes over two years (2019-2020). A total of 21 mosquito species were identified, with *Culex pipiens* s.l. being the most abundant and widespread species. The three ecological forms of *Cx. pipiens* were found and *Cx. pipiens pipiens* showed to be the most common in both green areas and cemeteries. The morphological identification was accompanied by molecular tools. Vitoria-Gasteiz exhibited the highest species richness, followed by Donostia and Bilbao. Mosquito abundance was significantly higher in green areas compared to cemeteries, and in Donostia and Bilbao compared to Vitoria-Gasteiz. The investigation of larval rearing sites highlighted the dominance of *Cx. pipiens* s.l., particularly in semi-artificial ponds, diverse water-holding containers (tyres and buckets), and drainage systems in green areas, whereas in cemeteries flowerpots comprised most of the larval abundance together with funeral urns. Seasonal activity exhibited varying peaks in mosquito abundance across different cities, with a notable increase occurring in July or August. Additionally, blood meal analysis revealed *Cx. pipiens* s.l. feeding on various common urban avian species.

The expansion of invasive mosquitoes throughout Europe has increased in recent decades. In northern Spain, *Aedes albopictus* was detected for the first time in 2014, and *Aedes japonicus* was detected in the three Basque provinces in 2020. The fourth study (**Study IV**) of his thesis aimed to evaluate the distribution of invasive mosquito species and their association with factors related to urbanization. In 2021, a total of 568 ovitraps were deployed from June to November in 113 sampling sites from 45 municipalities with > 10,000 inhabitants. Eggs were detected in 66.4% of the sampling sites and in 32.4% of the ovitraps distributed in the three provinces. The eggs from a selection of positive oviposition sticks, encompassing at least one stick from each positive ovitrap, were hatched following their life cycle until the adult stage. When egg hatching was not successful, PCR targeting the COI gene and sequencing of amplicons were carried out. *Aedes albopictus* and *Ae. japonicus* were widespread in the studied area, confirming their presence in 23 and 26 municipalities, respectively. Co-occurrence of both species was observed in 11 municipalities. The analysis of the presence of *Aedes* invasive mosquitoes and the degree of urbanization (urban, suburban, peri-urban) revealed that *Ae. albopictus* showed a 4.39 times higher probability of being found in suburban areas than in peri-urban areas, whereas *Ae. japonicus* had a higher probability of being found in peri-urban areas. Moreover, the presence of *Ae. albopictus* was significantly associated with municipalities with a higher population density (mean = 2,983 inh/km2), whereas *Ae. japonicus* was associated with

lower population density (mean = 1,590 inh/km2). The wide distribution of *Ae. albopictus* and *Ae. japonicus* observed confirmed the spread and establishment of these species in northern Spain. A new colonization area of *Ae. japonicus* in Europe was confirmed. Due to the potential impact of *Aedes* invasive mosquitoes on public health and according to our results, surveillance programs and control plans should be designed considering different urbanization gradients, types of environments, and population density.

Studies on mosquitoes are essential for comprehending their role in disease transmission and devising targeted, sustainable management strategies to mitigate associated risks. Mosquitoes in Europe represent a crucial facet of public health concern. As urbanization intensifies, these areas increasingly serve as vital habitats for diverse mosquito species, fostering breeding grounds and increasing the risk of disease transmission. The results obtained in this thesis highlights the advantages of mosquito sampling using various techniques to comprehensively characterise species composition and abundance. Information on the trophic preferences and biting behaviour of mosquitoes is also provided.

Laburpena

Eltxoak (Diptera: Culicidae) ingurune desberdinetan maiz aurkitzen diren Diptero odol-jaleak dira. Haien banaketa, ugaritasuna eta dinamika ekologikoa ulertzea ezinbestekoa da, bereziki urbanizazioa gero eta handiagoa den eta espezie inbaditzaileen areagotzearen testuinguruan. Doktorego tesi honek ezagutza-hutsune horri aurre egin nahi izan dio, Euskal Autonomi Erkidegoko eltxo-espeziei buruzko ikerketa integral bat eginez, ingurune eta antropizazio-maila ezberdinetan arreta jarriz. Helburu orokorra hirukoitza da: lehenik, Culicidae espezieak identifikatzea ingurune natural, landa-eremu, hiri-inguru eta hirietan zehar, haien presentzia, ugaritasuna eta aberastasuna moldatzen duten faktoreak aztertuz; bigarrena, *Aedes* spp. inbaditzaileen banaketa aztertzea antropizazio-maila ezberdinetako eremuetan eta horietan eragiten duten faktoreak ebaluatzea; eta hirugarrena, eltxo-espezieen elikadura ikertzea hiritik ingurune naturalerako gradiente batean. Helburuari aurre egiteko lau azterketa egin dira.

Hezeguneak ekosistema baliotsuak dira animalia espezie askorentzat, bereziki hegazti migratzaileentzat. Hori dela eta, animalia-espezieen eta eltxoen arteko elkarrekintzak paper garrantzitsua izan dezake patogenoen transmisioan. **I. Azterlanean**, bi hezegunetatik eltxoak bildu ziren metodologia desberdinak erabiliz eta morfologia klasikoa eta tresna molekularrak erabiliz identifikatu ziren. Guztira, 22 eltxo-espezietako 1529 eltxo ar eta eme harrapatu ziren, Gaixotasunen Kontrolerako eta Prebentziorako Zentroa (CDC) tranpak eta sareak erabilita. Oro har, espezierik ugariena *Coquillettidia richiardii* izan zen, ondoren *Aedes rusticus*, *Culex pipiens* s.l. eta *Culex modestus*, baina espezieen konposizioa aldatu egin zen ingurune urtarraren arabera. Odolez elikatuta zeuden eltxo emeen artean, 11 ornodun espezie ostalari—sei ugaztun eta bost hegazti— identifikatu ziren DNA *barcoding* erabiliz. Zortzi eltxo espezieren hazkuntza-tokiak bederatzi mikrohabitatetan zehaztu ziren, eta 11 eltxo espezie harrapatu ziren gizakien gainean jarrita. Hegaldi-aldia eltxo-espezieen artean aldatu egin zen, batzuk udaberrian eta beste batzuk udan.Aldi berean, moskitoen aniztasuna eta ugaritasuna ere ikertu ziren landa edo hiri periferietan kokatutako Animalien Babeserako hiru zentrotan (APC). Tesi honen bigarren ikerketan (**II. Azterlana**), APC bakoitzean lau CDC argi-tranpa jarri ziren (bi UV eta bi bonbilla gori estandar, bai CO2-arekin beitatuak eta ez beitatuak). Guztira 224 eltxo (8 espezie) bildu eta identifikatu ziren metodologia morfologiko zein molekularren bidez. *Culex pipiens*/*Cx. torrentium*-ek Culicidaeen % 76,8 osatzen zuten. Argi-tranpetan CO2-aren gehitzeak eraginkortasun txikiagoa izan zuen eltxoen bilketak hobetzeko, eta UV-argidun tranpak 2,2 aldiz eraginkorragoak izan ziren argi-tranpak baino. Eltxoen harrapaketak nabarmen handiagoak izan ziren uda hasieran.

III. Azterlana Euskal Autonomi Erkidegoko hiru hiri nagusietan egin zen, hirietako berdeguneetako eta hilerrietako eltxoen espezieen osaera, ugaritasuna, populazio dinamikak, larben habitatak eta ostalari-lehentasunak ikertzeko. CDC tranpak eta ur-bilketak erabili ziren bi urtetan (2019-2020) eltxoak biltzeko. Guztira 21 eltxo espezie identifikatu ziren, *Culex pipiens* s.l. espezie ugariena eta hedatuena izanik. *Cx. pipiens*en hiru forma ekologikoak aurkitu ziren *eta Cx. pipiens pipiens* berdeguneetan zein hilerrietan ohikoena zela aurkitu zen. Identifikazio morfologikoa tresna molekularrekin batera erabili zen. Gasteizek izan zuen espezie-aberastasun handiena, eta ondoren Donostiak eta Bilbok. Eltxoen ugaritasuna nabarmen handiagoa zen berdeguneetan hilerriekin alderatuta, eta Donostian eta Bilbon Gasteizekin alderatuta. Larben hazkuntza-tokien ikerketak *Cx. pipiens* s.l.- ren nagusitasuna nabarmendu zuen batez ere urmael erdi-artifizialetan, ura jasotzeko hainbat ontzitan (pneumatikoak eta kuboak) eta berdeguneetako drainatze-sistemetan. Hilerrietan, berriz, loreontziek larba-ugaritasun gehiena zuten hileta-urnekin batera. Urtaroko jarduerak eltxoen ugaritasunaren gailur desberdinak izan zituen hiri ezberdinetan, uztailean edo abuztuan igoera nabarmena izan zelarik. Gainera, odol-otorduaren analisiak *Cx. pipiens* s.l. hiriko hegazti espezie arruntez elikatzen zela agerian utzi zuen.

Europan zehar eltxo inbaditzaileen hedapena areagotu egin da azken hamarkadetan. Espainia iparraldean 2014an detektatu zen lehen aldiz *Aedes albopictus*, eta 2020an *Aedes japonicus* hiru euskal probintzietan. Tesiaren laugarren ikerketak (**IV. Azterketa**) eltxo espezie inbaditzaileen banaketa eta urbanizazioarekin zerikusia duten faktoreekin zuten lotura ebaluatzea izan zuen helburu. 2021ean, 568 ovitranpa zabaldu ziren ekainetik azarora bitartean > 10.000 biztanleko 45 udalerritako 113 laginketa gunetan. Arrautzak laginketa-guneen % 66,4an eta hiru lurraldeetan banatutako obitranpen % 32,4an detektatu ziren. Obiposizio makil positibo batzuen arrautzak (obitranpa positibo bakoitzeko gutxienez makil bat) beren bizi-zikloari jarraituz helduen fasera arte garatu ziren laborategian. Arrautzen eklosioak arrakastarik izan ez zuenean, COI geneari zuzendutako PCR eta anplikoien sekuentziazioa burutu ziren. *Aedes albopictus* eta *Ae. japonicus* oso hedatuta zeuden aztertutako eremuan. 23 eta 26 udalerritan, hurrenez hurren, haien presentzia baieztatu zen eta 11 udalerritan bi espezieen presentzia detektatu zen. *Aedes* eltxo inbaditzaileen presentziaren eta urbanizazio-mailaren (hiri, hiri-ingurune eta hiri-periferia) aztertzeak agerian utzi zuen *Ae. albopictus* aurkitzeko aukera 4,39 aldiz handiagoa izan zela hiri-inguruetan hiri-periferietan baino. *Aedes japonicus*ek, berriz, hiri-periferiako eremuetan aurkitzeko probabilitate handiagoa zuen. Gainera, *Ae. albopictus*ren presentzia biztanleria dentsitate handiagoko udalerriekin (batezbestekoa = 2,983 hab/km2) nabarmen lotu zen, *Ae. japonicus* populazio-dentsitate baxuagoarekin lotu zen bitartean (batezbestekoa = 1.590 hab/km2). *Ae. albopictus* eta *Ae. japonicus*en banaketa zabalak espezie horiek Espainiako iparraldean zabaldu eta ezarri zirela baieztatu zuen. *Aedes japonicus* espeziearen kolonizazio eremu berri bat baieztatu zen Europan. *Aedes* eltxo inbaditzaileak osasun publikoan izan dezakeen eragina dela eta eta gure emaitzen arabera, zaintza-programak eta kontrol-planak urbanizazio-gradiente, ingurune mota eta populazio-dentsitate desberdinak kontuan hartuta diseinatu behar dira.

Eltxoei buruzko ikerketak ezinbestekoak dira gaixotasunen transmisioan duten zeregina ulertzeko eta kudeatzeko estrategia bideratu eta iraunkorrak asmatzeko, lotutako arriskuak arintzeko. Europan bizi diren eltxoak osasun publikoaren kezka garrantzitsua dira. Urbanizazioa areagotu ahala, eremu hauek eltxo-espezie askorentzat ezinbesteko habitat gisa balio dute, ugalketa-lekuak sustatuz eta gaixotasunak transmititzeko arriskua areagotuz. Tesi honetan lortutako emaitzek eltxoen laginketaren abantailak nabarmentzen dituzte hainbat teknika erabiliz espezieen osaera eta ugaritasuna modu integralean karakterizatzeko. Eltxoen lehentasun trofikoei eta hozka-jokabideei buruzko informazioa ere ematen da.

Resumen

Los mosquitos (Diptera: Culicidae) son dípteros hematófagos que se pueden encontrar en diferentes ambientes. Comprender su distribución, abundancia y dinámica ecológica es esencial particularmente en el contexto de una creciente aparición de especies invasoras. Esta tesis doctoral ha tenido como objetivo abordar esta falta de conocimiento mediante la realización de una investigación exhaustiva sobre las especies de mosquitoes en el País Vasco, en diferentes ambientes y grados de antropización del paisaje. El objetivo general consta de tres partes: en primer lugar, identificar las especies de Culicidae en entornos naturales, rurales, periurbanos y urbanos, explorando los factores que afectan a su presencia, abundancia y riqueza; en segundo lugar, estudiar la distribución de especies invasoras de *Aedes* spp. en diversos grados de antropización y evaluar los factores que influyen en su presencia; por último, investigar los patrones de alimentación de las especies de mosquitos a lo largo de un gradiente urbano-natural. Para abordar este objetivo se han realizado cuatro estudios.

Los humedales son ecosistemas valiosos, en particular para las aves migratorias. Por lo tanto, las interacciones entre estas especies y los mosquitos pueden desempeñar un papel crítico en la transmisión de agentes patógenos. En el **Estudio I**, durante 2018-2019, se capturaron mosquitos en dos ecosistemas acuáticos utilizando diferentes metodologías, y se identificaron mediante morfología clásica y/o métodos moleculares. Se capturó un total de 1529 machos y hembras de 22 especies nativas de mosquitos mediante trampas CDC y manga. En general, la especie más abundante fue *Coquillettidia richiardii*, seguida de *Aedes rusticus*, *Culex pipiens* s.l. y *Culex modestus*, pero la composición de especies varió en función del medio acuático En los mosquitos hembra alimentados con sangre, se identificó ADN de 11 especies de hospedadores vertebrados -seis mamíferos y cinco aves. También se caracterizaron los lugares de cría de ocho especies de mosquitos en nueve microhábitats, y mediante "*human landing collection*" se capturaron 11 especies de mosquitos que se posaban en el operador. El periodo de vuelo varió entre las especies de mosquitos, alcanzando algunas su máximo en primavera y otras en verano.

Simultáneamente, también se investigó la diversidad y abundancia de mosquitos en tres centros de protección animal situados en zonas rurales o suburbanas. En este segundo estudio de esta tesis (**Estudio II**), en cada centro se colocaron cuatro trampas CDC (dos con luz ultravioleta y dos de bombilla incandescente estándar, ambos tipos cebadas y no cebadas con CO_2). Se recogió un total de 224 mosquitos (8 especies), que se identificaron mediante métodos morfológicos y moleculares. *Culex pipiens/Cx. torrentium* constituyeron el 76,8% de los Culicidae capturados. El aporte de CO_2 en las trampas de luz no mejoró las capturas, y las trampas de luz

ultravioleta fueron 2,2 veces más eficaces que las de luz incandescente. Las capturas de mosquitos fueron significativamente mayores al principio del verano.

Se realizó un tercer estudio (**Estudio III**) en espacios verdes urbanos y en cementerios de las tres principales ciudades del País Vasco, con el objetivo de investigar la composición de Culicidae, su abundancia, estacionalidad, características de los hábitats larvarios y las preferencias de hospedadores. Se utilizaron trampas CDC y el estudio de focos de cría para recolectar mosquitos durante 2019 y 2020. Se identificaron un total de 21 especies de mosquitos, siendo *Culex pipiens* s.l. la especie más abundante. Se encontraron las tres formas ecológicas de *Cx. pipiens*, y *Cx. pipiens pipiens* mostró ser la más común tanto en zonas verdes como en cementerios. La identificación morfológica se acompañó de métodos moleculares. Vitoria-Gasteiz presentó la mayor riqueza de especies, seguida de Donostia y de Bilbao. La abundancia de mosquitos fue significativamente mayor en las zonas verdes en comparación con los cementerios, y en Donostia y Bilbao en comparación con Vitoria-Gasteiz. La investigación de los lugares de cría de larvas puso de manifiesto el predominio de *Cx. pipiens* s.l., en estanques semiartificiales, diversos recipientes con agua (neumáticos y cubos) y en sistemas de drenaje en zonas verdes, mientras que en los cementerios las macetas constituían la mayor parte de la abundancia de larvas junto con las urnas funerarias. La actividad estacional mostró picos variables en la abundancia de mosquitos en las distintas ciudades, con un aumento notable en julio o agosto. Además, el análisis del ADN del hospedador en las hembras alimentadas de sangre reveló que las preferencias de alimentación de *Cx. pipiens* s.l. eran las especies comunes de aves urbanas.

La expansión de mosquitos invasores por Europa ha aumentado en las últimas décadas. En el País Vasco, *Aedes albopictus* se detectó por primera vez en 2014, y *Aedes japonicus* se detectó en las tres provincias vascas en 2020. El cuarto estudio de esta tesis (**Estudio IV**) ha tenido como objetivo evaluar la distribución de las especies de mosquitos invasores y su asociación con factores relacionados con la urbanización. En 2021, se desplegaron un total de 568 ovitrampas de junio a noviembre en 113 puntos de muestreo de 45 municipios con > 10.000 habitantes. Se detectaron huevos en el 66,4% de los puntos de muestreo y en el 32,4% de las ovitrampas distribuidas en las tres provincias. Los huevos procedentes de una selección de tablillas de oviposición positivas, que abarcaba al menos una tablilla de cada ovitrampa positiva, se pusieron a mudar hasta la fase adulta. Cuando la eclosión de los huevos no tuvo éxito, se llevó a cabo la técnica PCR (gen COI) y la secuenciación de los amplicones. *Aedes albopictus* y *Ae. japonicus* mostraron estar ampliamente distribuidos, confirmándose su presencia en 23 y 26 municipios, respectivamente. La coocurrencia de ambas especies se observó en 11 municipios. El análisis de la presencia de mosquitos invasores *Aedes* y el grado de urbanización (urbano, suburbano, periurbano) reveló que *Ae. albopictus* presentaba una probabilidad 4,39 veces mayor de encontrarse en zonas suburbanas que en zonas periurbanas, mientras que *Ae. japonicus* tenía una probabilidad mayor de encontrarse en zonas periurbanas. Además, la presencia de *Ae. albopictus* se asoció significativamente a municipios con una mayor densidad de población (media = 2.983 hab/km^2), mientras que *Ae. japonicus* se asoció a una menor densidad de población (media = 1.590 hab/km^2). La amplia distribución de *Ae. albopictus* y *Ae. japonicus* observada confirmó la expansión y establecimiento de estas especies en el País Vasco. Se confirmó una nueva zona de colonización de Ae. *japonicus* en Europa. Debido al potencial impacto de los mosquitos invasores *Aedes* en la Salud Pública y de acuerdo con nuestros resultados, los programas de vigilancia y los planes de control deberían diseñarse considerando diferentes gradientes de urbanización, tipos de ambientes y densidad de población.

Los estudios sobre las especies de mosquitos presentes en una determinada zona son esenciales para comprender su papel en la posible transmisión de enfermedades y, poder diseñar estrategias de gestión específicas y sostenibles para mitigar los riesgos asociados a su presencia. Los mosquitos que habitan en Europa representan una faceta crucial de la salud pública. A medida que se intensifica la urbanización, estas zonas sirven cada vez más como hábitats para diversas especies de mosquitos, fomentando los focos de cría y aumentando, por consiguiente, el riesgo de transmisión de enfermedades. Los resultados obtenidos en esta tesis destacan las ventajas del muestreo de mosquitos mediante diversas técnicas para caracterizar exhaustivamente la composición y abundancia de las especies de mosquitos. También se aporta información sobre las preferencias tróficas de las especies halladas, así como su comportamiento respecto a la picadura.

I. General Introduction

I.1. Culicidae

Mosquitoes are a diverse group of insects that have great relevance in Public Health, not only because of their biting nuisance but also because of their role as vectors of diseases that cause millions of deaths around the world (Tolle, 2009). A total of 3,614 species of mosquitoes are currently recognized (Harbach, 2023). They are found all over the world in almost all types of ecological zones, being absent only from Antarctica and some islands (Mehlhorn, 2016).

Mosquitoes are ecologically beneficial to biodiversity, due to their role in food chains and as pollinators and only some species are sources of threats to humans and animals. The different mosquito species have adapted to different environments having different strategies and preferences for feeding and oviposition (Burkett-Cadena, 2013).

Human activities, such as global trade, travelling and urbanization, have a great impact on the establishment and spread of vectors and vector-borne pathogens (Medlock et al., 2012b). However, climatic suitability is a major factor for the settlement and establishment of invasive mosquito species. In fact, some of these species show great plasticity and have adapted to temperate climates therefore rapidly spreading over the last decades (Randolph and Rogers, 2010). There are about 20 invasive species that have significantly extended their distribution range in recent years (ECDC, 2012), such as *Aedes albopictus* (Aranda et al., 2006; Cunze et al., 2016; Schaffner et al., 2003; Scholte et al., 2007), *Aedes japonicus* (Koban et al., 2019; Montarsi et al., 2019; Schaffner et al., 2003; Versteirt et al., 2009; Werner et al., 2012) or *Aedes koreicus* in Europe (Kalan et al., 2017; Kurucz et al., 2016; Marini et al., 2019; Versteirt et al., 2014; Werner et al., 2016), and *Aedes aegypti* and *Aedes albopictus* in America (Moore and Mitchell, 1997). Other mosquito species like *Aedes atropalpus* (Giunti et al., 2023b) and *Culex quinquefasciatus* have also been detected in Europe and in the Hawaiian Islands (Lapointe, 2008) respectively.

The great risk of the establishment of these invasive species is the emergence and re-emergence of diseases, that together with globalization may lead to serious human outbreaks of diseases in countries where these exotic mosquito species are absent (Brugueras et al., 2020). Moreover, climate change will also affect the incidence of these vector-borne pathogens, as the conditions will be more suitable for some mosquito species (Iwamura et al., 2020).

Blood-feeding is an essential requirement in the life cycle of mosquitoes. Usually, they feed on a wide range of animals, however, some show a preference for certain hosts. Besides, finding a host also depends on their availability in the environment. Therefore, many mosquitoes are opportunistic feeders and feed on the first host they find. This opportunistic behaviour is one of the causes of pathogen transmission, thus understanding their feeding patterns is of great importance to predict and control zoonosis (Takken and Verhulst, 2012).

I.1.1. Taxonomy and morphology

The family Culicidae, derived from *culex*, the Latin name for “gnat,” is a monophyletic taxon that belongs to the order Diptera (the “two-winged” insects), suborder Nematocera, and infraorder Culicomorpha (Foster and Walker, 2019) (Figure 1)

Phylum Arthropoda Latreille, 1829
Subphylum Hexapoda Latreille, 1825
Clase Insecta Linnaeus, 1758
Subclase: Holometabola Sharp, 1898
Orden Diptera Linnaeus, 1758
Suborden Nematocera Schiner, 1862
Infraorden Culicomorpha Hennig, 1948
Familia Culicidae Meigen, 1818

Figure 1. Classification of the Culicidae family based on Wilkerson et al. (2015)

The Culicidae family has two subfamilies: Anophelinae and Culicinae. The subfamily Anophelinae comprises 476 species, of which many of them belong to sibling species complexes and are awaiting formal names. This subfamily includes three genera: *Anopheles*, *Bironella,* and *Chagasia*. Of these three genera, the *Anopheles* genus contains the majority of the anopheline species, and they are the vectors, among others, of human malaria, an illness caused by *Plasmodium* parasites (Harbach, 2007). The Culicinae family comprises 3,119 species and is divided into 11 tribes and 38 genera. The genus *Aedes*, belonging to the Aedini tribe, is the largest containing nearly two-thirds of the species (1,216 species) and moreover, many of these species are important vectors of pathogens (Wilkerson et al., 2021). Regarding the Aedini tribe, there has been controversy about the classification of this group. Most of the species among this tribe belong to the genus *Aedes*, however, some phylogenetic studies reordered 74 genera within this group (Harbach, 2007), thus, complicating the classification of the Aedini tribe. Wilkerson et al. (2015) revised the classification used before the year 2000 placing all the species of the Aedini tribe in 10 genera (*Aedes, Armigeres, Eretmapodites, Haemogogus, Heizmannia,* *Opifex, Psorophora, Udaya, Verrallina and Zeugnomyia*), thus reducing the number of species within the genus *Aedes*.

Mosquitoes are slender, long-legged, two-winded insects usually small, between 3-6 mm in length (Service, 2012). The main characteristic of a mosquito is its specialised mouthpart called proboscis. The proboscis is well adapted to pierce the skin and suck the blood from its host. The proboscis projects forward together with the maxillary palps, that comprises labium and stylets, and is longer than the thorax (Becker et al., 2020). The stylet bundle is held together by the labium when the mosquito is not feeding and consists of six long thin stylets: the labroepipharynx, hypopharynx, two maxillae, and two mandibles. To break the skin and let the other stylets pass, the mandibles are sharply pointed (Clements, 2000).

They have like any other insects, three body parts: the head, the thorax, and the abdomen. Each one of these parts is as well divided into different segments. In the head, they have the sensory centre, and is mainly composed of their two large compound eyes (Burkett-Cadena, 2013). The thorax is located between the head and the abdomen, and it is composed of three segments: prothorax, mesothorax and metathorax. A pair of spiracles are in the mesothorax and metathorax. The scutum is the principal dorsal area of the thorax. The scutum is followed by the scutellum (Becker et al., 2020).

There are three pairs of legs on an adult, one pair on each thoracic segment. A leg is composed of six different segments: the coxa, trochanter, femur, tibia, tarsus and pretarsus. The tarsus is divided into five tarsomeres, and the last tarsomere ends with the pretarsus (Becker et al., 2020). The pretarsus is composed of a pair of claws or ungues and the empodium (Service, 2012). Wings are also located in the thoracic region; they are large and narrow. The halteres are the metathoracic wings which are modified to form small vibrating organs that have the function of controlling the equilibrium during the flight (Burkett-Cadena, 2013).

The abdomen of the mosquitoes consists of 10 segments, but only the first 7 or 8 are clearly visible (Service, 2012). The last abdominal segment ends with the genitalia. In females, it is composed of the cerci, while male genitalia are composed of a pair of lateral claspers, and the gonocoxite with the gonostylus (Becker et al., 2020). The abdomen of the unfed females is slender and thin, but after taking a blood-meal, the abdomen distends significantly. After the digestion of the blood, the eggs are developed, and the abdomen's colouring changes from red to white (Service, 2012).

Mosquito larvae are differentiated from other aquatic insects as they have a distinct head that has mouth brushes and antennae, a distended thorax that is wider than the head and the abdomen, absence of legs, and have either a pair of respiratory openings (subfamily Anophelinae) or an elongated siphon (subfamily Culicinae) borne near the end of the abdomen (Harbach, 2007). In fact, their body is divided into three parts: the completely sclerotised head, the thorax and the abdomen that consists of ten segments (Burkett-Cadena, 2013).

The body of the pupa is divided into two parts: the cephalothorax and the abdomen. The abdomen is articulated and usually flexed under the cephalothorax; its function is to propel the pupa while swimming. In fact, mosquito pupae are quick and can dive rapidly from the water surface when disturbed. However, they are usually found on the water surface with their respiratory trumpets in contact with the air (Becker et al., 2020).

I.1.2. Life cycle

Mosquitoes are holometabolous insects; therefore, they undergo a complete metamorphosis. It is biological cycle consists of four different stages: larva (four instars), pupa, adult (male and female) and egg. Adult mosquitoes are terrestrial, whereas the rest of the forms are aquatic (Figure 2) (Clements, 2000).

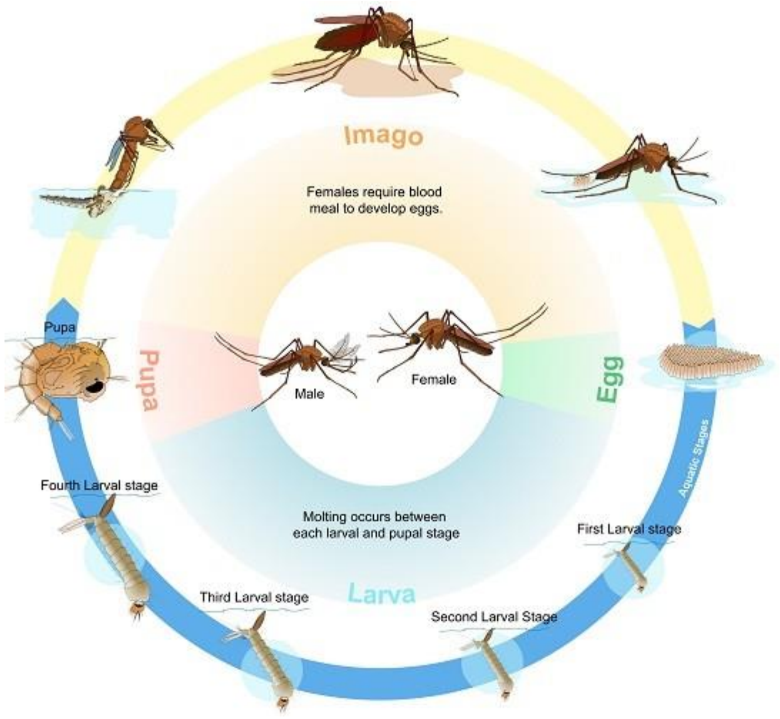

Figure 2. Life Cycle of the mosquito.

Diagram by Mariana Ruiz Villareal

Females can lay between 30 to 300 eggs. These eggs, depending on the genus and species, can either be laid on the water surface, on wet ground or the edge of water bodies. Moreover, they can be laid individually (e.g., *Aedes, Anopheles*) or grouped in egg rafts (e.g., *Culex*). After eggs are laid on the water surface, they usually hatch quickly after the oviposition, although the *Aedes* eggs deposited directly on the ground can remain inactive for up to a year or even several years if they are not submerged in water. Regarding anopheline mosquitoes, they lay their eggs individually on the water surface. In fact, these eggs have a special adaptation for floating, characterized by float-like structures present on both sides (Service, 2012).

The larvae hatch directly into the water, where they will develop and feed. The development of the larva depends on temperature and food availability (Becker et al., 2020). The temperature of the water is key in the hatching process, as the growth and development of the larva occur within a temperature range. If the hatching is during cold months, the development of the larvae would take longer. In fact, depending on the species there is a lower and upper lethal temperature and within this range, the rate of growth is positively correlated with temperature (Clements, 2000).

The larval phase is composed of four instars, they grow continuously throughout each instar from about 2 mm in the first instar to around 10 mm in length in the fourth and last instar. The larval growth is a secondary sexual character as adult females are normally heavier than males (Becker et al., 2020; Clements, 2000).

The last larval instar then produces the pupa. During this last aquatic phase, the pupa does not feed, and the metamorphosis takes place creating the adult form. Even though these two stages are aquatic, they need air to breathe that they take from the water surface therefore they can live in water with low oxygen levels or polluted (Service, 2012). However, some genera such as *Coquillettidia* have a modified siphon able to pierce parts of aquatic plants that are submerged in order to use the oxygen from their aerenchyma. The pupae from the *Coquillettidia* genus, which are fixed to the plant tissues underwater, have to swim to the water surface before the emergence of the adult form (Bosak and Crans, 2002).

The adult form emerges from the pupa during the ecdysis process in which the skin is shredded. When the metamorphosis is complete the gas forces the cuticle of the pupa to split along the ecdysial line, and therefore the adult emerges slowly. The emergence is cautious in order to avoid contact with the water surface, until its legs are freed. During this phase, the mosquito is highly vulnerable to strong winds and predators (Becker et al., 2020). For a short period of time, the adult rests on the water surface until it flies away. Moreover, males usually emerge before the females, both in laboratory and in the wild, as the duration of the larval and pupal phases of males are slightly shorter, in accordance as well with their differences in size (Clements, 2000).

Male mosquitoes after their emergence are not sexually mature, as they need to rotate 180º their hypopygium before they are able to mate, which takes about a day to complete. In fact, usually, the males emerge one or two days before the females to be sexually mature when the females emerge. During the larval phase, sexually dimorphic characters exist as the male development is shortened, thus making adult males smaller in size in comparison with females. These differences in size are useful for the separation of sexes (Clements, 1999).

Mating in mosquitoes takes place during flight, males form swarms that the females go into. This swarm is formed to attract conspecific females and for that reason, they perform an oscillation pattern called "dancing flight". A swarm can be composed of a few male mosquitoes or up to several thousands. It is usually formed in the evenings and mornings over a marker at low light intensities. When a female joins the swarm, it is immediately seized by a male (Becker et al., 2020). It takes normally about less than a minute for the male to deposit the sperm, and females store it in the spermathecae. They store the sperm to fertilise several batches of eggs for the rest of its life, therefore not needing to copulate anymore. On the other hand, males mate many times (Clements, 2000). Depending on the species, some can mate in confined spaces, like *Cx. pipiens* form *molestus*, requiring no nuptial flight, these are called stenogamous species, while others mate in open spaces in the air like *Cx. pipiens* form *pipiens*, which are called eurygamous (Kim et al., 2018). Usually, female mosquitoes are anautogenous, laying their eggs after taking a blood-meal (Clements, 2000), while other species like *Cx. pipiens* form *molestus* are autogenous, which is the ability to lay their eggs before taking a blood-meal (Clements, 2000; Haba and McBride, 2022).

I.1.3. Habitat and nutrition

Each mosquito species has different requirements and has adapted to specific habitats. Physicochemical variables of the water, such as temperature and hydrogen ion concentration (pH) (Amerasinghe et al., 1995), as well as the presence of potential predators, vegetation (Shililu et al., 2003), and degree of organic matter (Lorenz et al., 2013) are key for the selection of the oviposition sites (Mereta et al., 2013; Muturi et al., 2008). For example, *Aedes vexans* larvae are usually found in rainwater pools, but not in tree holes or man-made containers. On the other site, *Aedes geniculatus* larvae are typically found in tree holes but they can be found also in man-made containers (Becker et al., 2020). *Aedes albopictus* and *Ae. aegypti*, have adapted and man-made containers such as used tyres, cans or flowerpots are usual habitats for these mosquitoes (Medlock et al., 2012a). Moreover, they can live in a wide range of habitats, but not in turbulent or fast-flowing water (Burkett-Cadena, 2013).

Adult mosquitoes also need and thrive in specific environments. For example, *Aedes caspius* and *Aedes detritus* are commonly found in coastal areas, such as wetlands and marshes. In fact, *Ae. detritus* larvae can be found in water with high concentrations of salt (Hawkes et al., 2020). Other mosquitoes, such as *Ae. aegypti* or *Ae. albopictus*, have adapted and are very common in urban and suburban areas (Medlock et al., 2012b). However, *Ae. japonicus* is considered a more rural mosquito (Tanaka et al., 1979).

Larvae feed on microorganisms, protozoa, algae, invertebrates, and detritus. Depending on their feeding behaviour larvae can be classified into three categories: filter or suspension feeders, browsers, and predators (Becker et al., 2020). The filter feeder larvae generate currents of water by beating their head brushes toward their preoral cavity so that the food particles move toward their mouth. They either move slowly in the water or hang on the water surface filtering particles; the latter is the most common one (Merritt et al., 1992). Most *Anopheles* larvae are found feeding by filtering in the interface of water and air, whereas many *Culex* and *Culiseta* larvae feed in the water column and *Mansonia* and *Coquillettidia* species feed around plant stems and roots. Their major food resource is detritus and microorganisms (Clements, 2000; Merritt et al., 1992). Also, they usually are not very restrictive in their food intake, but the size of the particles is generally less than 50 µm. As for the browser feeder larvae, they collect nutrients either by scraping, shredding, or resuspending particles, microorganisms, algae, and protozoa they find. They even can bite with their mouth parts small portions of plants and dead invertebrates (Merritt et al., 1992). Many *Aedes* larvae are browsers, for example *Ae. atropalpus* collects food that is adhered to surfaces of submerged plants and minerals. The predatory larvae feed upon other insects, and often on other mosquito larvae, this behaviour has not been reported in Europe, but it is observed in some larvae of *Toxorynchites*, *Aedes*, *Psorophora* and *Culex* (Becker et al., 2020; Clements, 2000). Moreover, larval growth rate decreases if the food available is below a specific range. Thus, some critical factors are the larval density and the amount of food available in the water. Intraspecific competition has been observed, both in the field and in the laboratory, due to high larval density thus resulting in a longer developing time, and a reduction in the pupation success and weight. Moreover, in some species, if there is a high larval density this leads to cannibalism in larvae that are usually non-predatory (Merritt et al., 1992).

The major food resource in adult mosquitoes is plant juices. In fact, floral nectar is the most common one, but they also take sugar meals from decaying fruits, honeydew, and intact vegetative tissues. This is the only energy source male mosquitoes have; however, it is also an important source of energy for females. Females also need to ingest blood-meals from animals, specifically the proteins necessary to produce the eggs (Clements, 2000). Only a few autogenous species, like *Cx. pipiens* form *molestus*, can produce eggs without a blood-meal (Weitzel et al., 2009). Most species feed on mammals and birds, but some species also feed on reptiles, amphibians, and fishes (Tempelis, 1975). In fact, mosquitoes usually take several blood-meals, this behaviour is due to taking only small portions while hosts are defending themselves (Edman and Kale, 1971). Moreover, they ingest two to four times more blood than their own weight (Nayar and Sauerman, 1977). Blood-meals are ingested with their specialised mouthparts. When the female is feeding, they inject saliva into the host to stop the coagulation of the blood. Therefore, this behaviour makes mosquitoes so important to public and veterinary health, as they can ingest pathogens from an infected host and then inject them in a following blood-meal into another host (Clements, 2000). Several pathogens have evolved to exploit this route accumulating in the salivary gland of the mosquito (Pimenta et al., 1994; Raquin and Lambrechts, 2017). Consequently, a mosquito is vector on the account to its capacity to transmit pathogens thus causing human and animal diseases (Clements, 2012).

Depending on the species, feeding, and resting behaviour are different. Some mosquitoes are endophagic and others are exophagic depending on their preferences to feed indoors or outdoors, respectively. After taking the blood-meal some prefer resting indoors (endophilic), while others on the contrary like to rest outdoors (exophilic). Normally, *Ae. albopictus* females like to bite and rest outdoors. On the contrary, *Cx. pipiens* s.l. usually prefer to feed and rest indoors (ECDC, 2014). Moreover, they also can have host feeding preferences, for example, some like to feed on birds and others on humans, the former is called ornithophilic and the latter anthropophilic species (Service, 2012).

I.1.4. Mosquito phenology

Mosquitoes show seasonal dynamics driven by environmental conditions that affect their activity, development, survival, and overwintering behaviour. These periods of activity are regulated by different factors in which the natural cycle of light and darkness, as well as climatic factors are involved. It is difficult to decipher how these factors affect individually the seasonality dynamics as they are all associated (Clements, 1999).

Mosquitoes have periods of activity and inactivity that are correlated with the natural cycle of light and darkness. Some species are active during the twilight or during the hours of darkness, while others on the contrary are active during the daylight. Moreover, they can even exhibit different behaviour, such as mating, feeding or oviposition, at different time of the day (Clements, 1999). Diapause is induced by the shortening of the length of the day (Fyie et al., 2021) as they have circadian clocks, like the ones in mammals and other animals (Young and Kay, 2001). Furthermore, urban light pollution caused by '*artificial light at night'* inhibits the diapause, thus being active for a longer period during the year (Fyie et al., 2021).

As insects cannot regulate their internal temperature, changes in climatic conditions play a fundamental role in their abundance, distribution and even transmission of vector-borne pathogens (Kilpatrick and Randolph, 2012). In fact, climatic factors, such as temperature, humidity, and rain, are key for the development of mosquitoes. The changes in climatic conditions affect mosquito populations from small time scales like daily weather, medium scales like seasonal changes, up to a longer time scales as with climate change (Campbell-Lendrum et al., 2015). The influence that climate change has in the present and future distribution of mosquitoes will be reviewed below.

Temperature can either be inhibitory or permissive, in other words, each species has an optimum temperature range in which they can develop and reproduce. Water temperature is key in the development of the larvae, shortening the cycle as the temperature rises. However, the cycle is lengthened when the temperature comes closer to becoming lethal for the larvae (Clements, 2000; Paaijmans et al., 2010).

Water is an essential requirement in the life cycle of the mosquito, in fact, precipitations create the oviposition habitats, such as small pools of water that become enriched and that are needed by the female mosquitoes to lay their eggs. Therefore, rainfall and humidity are key in their population dynamics. Rainfall can affect the life cycle either by increasing the humidity or by altering the abundance and type of aquatic habitats available for the oviposition of the eggs and following the development of the larvae (Shaman and Day, 2007). For example, heavy rainfall can flush larval habitat, thus reducing the productivity of adult mosquitoes. Particularly, *Ae. aegypti* resisted better the rainfall flushing effect than *Cx. pipiens* (Koenraadt and Harrington, 2008). Moreover, in periods of drought conditions, the aquatic ecosystem of the predators and competitors of mosquito larvae can be disrupted, thus allowing larvae to develop and emerge as adults (Poh et al., 2019). Besides, humidity affects enhancing the mosquito flight activity and host-seeking behaviour, as rainfall increases the near-surface humidity levels (Shaman and Day, 2007).

Besides, depending on the region, tropical or temperate, mosquitoes have different survival strategies. In the tropics, the main difficulty is the survival during the dry season (Minakawa et al., 2001) while in temperate regions it is hibernation (Mori et al., 1981). Mosquitoes have developed two main strategies, either to reproduce continuously throughout the year or hibernate in the different stages (Minakawa et al., 2001; Reinhold et al., 2018).

Mosquitoes in the temperate region can either remain in the egg, larval or adult stage for overwintering. This behaviour is species-specific and in fact some species can overwinter in more than one stage. The duration of the hibernation is driven by several factors among which the latitude and hydrological conditions are included (Becker et al., 2020). Most *Aedes* species in temperate regions hibernate in the egg form. These eggs do not hatch until they find suitable climatic and hydrological conditions. This diapause makes the egg highly resistant to desiccation and low temperatures (Gillett, 1955).

Other mosquitoes overwinter in their larval or pupal stage, usually in the third or fourth larval instar. These larvae can survive as their metabolism is reduced and their development is delayed. Moreover, they can survive for a couple of days in their breeding sites with the water surface frozen, but during severe winters the mortality rate may be very high (Becker et al., 2020). Larvae of the *Coquillettidia* genus, as they obtain their oxygen from plants, are not susceptible to longer frost periods (Bosak and Crans, 2002).

Despite that, almost all the mosquito species overwinter as adult females. They usually hibernate sheltering frost-free sites such as caves, stables, cellars, canals, or earth burrows. They stay in these locations from autumn until the temperatures rises in spring. For surviving, the females usually consume what is left of the lipids from their larval body and they also continuously feed on plant juices throughout the autumn to increase their lipid reserves for the diapause (Becker et al., 2020).

Other strategy that mosquitoes have developed is the number of generations per year. Some mosquito species are univoltine, which means they only have one generation every year. For example, *Aedes rusticus* is a univoltine species, larvae hatch after heavy rainfalls in autumn and hibernate in the second and third instar and adults emerge in early spring (Becker et al., 2020; Medlock and Vaux, 2015b). Other species have two generations per year, these mosquitoes are bivoltine, like for example *Anopheles claviger* s.l. (Hawkes et al., 2020). While others are multivoltine, being active all year long like *Ae. aegypti* and *Ae. albopictus* in the tropics (Reinhold et al., 2018).

I.1.5. Mosquitoes of Europe

I.1.5.1. Invasive mosquitoes

It is important to know which species of mosquitoes, as well as their abundance, are present in each region to evaluate the risk of transmission of pathogens. The risk of transmission of mosquito-borne diseases in Europe is increasing due to globalization, climate change, as well as the environmental changes made by humans (Brugueras et al., 2020).

Moreover, these threats are mainly generated by the introduction of new mosquito species from distant countries. Invasive alien species are those whose occurrence in a region can be attributed to intentional or unintentional human actions that provided them the ability to cross biogeographical limits (Pyšek et al., 2020). Invasive mosquito species (IMS) can affect or likely jeopardize the economy, environment, and human health (Schaffner et al., 2013). In fact, IMS are currently of great interest throughout the globe, as they act as vectors of many pathogens (Schaffner et al., 2013).

In the western Palaearctic region, there are 141 native mosquito species and six invasive species, making a total of 147 species in total (Robert et al., 2019). The six IMS described in Europe are *Ae. aegypti*, *Ae. albopictus*, *Ae. japonicus*, *Ae. koreicus, Ae. triseriatus* and *Ae. atropalpus*. Sixty-five species of mosquitoes have been described in Spain, of which three are invasive (Bueno-Marí et al., 2012; Eritja et al., 2019): *Ae. aegypti*, *Ae. albopictus* and more recently *Ae. japonicus* (Eritja et al., 2019).

Regarding IMS present in Europe, *Ae. aegypti*, also known as the “yellow fever mosquito” is a vector of several arboviruses causing yellow fever, dengue, chikungunya or Zika diseases (Wint et al., 2022). This species has its origins in the sub-Saharan Africa where probably bred in tree holes and fed on non-human animals. Later, *Ae. aegypti* adapted and started to lay their eggs in human made structures or containers such as flowerpots and being anthropophilic. It is still unknown if this “domestication” was prior or occurred as it colonised new areas through sailing ships from Africa to the Mediterranean Basin and America. *Aedes aegypti* was present in Europe until mid-20th century when it was eradicated (Powell and Tabachnick, 2013). In Spain, it was present until 1953, however, the reasons for its disappearance are not clear. It has been theorised that it could be due to thermic tolerance and the intensive mosquito control and eradication campaigns with the use of di-cloro-difenil-tricloroetano (DDT) (Bueno-Marí et al., 2012).

Aedes aegypti has been so far detected in Madeira, Georgia, some southern parts of Russia and north-eastern Turkey (Figure 3). Moreover, it was introduced in the Netherlands, in a company importing used tyres in 2010 (Scholte et al., 2010), and later in 2016, at airport facilities arriving in flights from overseas (Ibañez-Justicia et al., 2017). However, these introductions were eradicated by applying the necessary control measures. Moreover, in 2017 it was detected in Spain, in the island of Fuerteventura, but due to the entomological Surveillance Programme implemented in Spain in possible *Points of Entry*, this introduction was eliminated (Barceló et al., 2022). More recently, *Ae. aegypti* has been detected in La Palma (2022) and Tenerife (2022 and 2023) islands (Centro de Coordinación de Alertas y Emergencias Sanitarias, 2023a).

It is very important to keep the surveillance on this species, as it has the capacity to transmit several pathogens of medical and veterinary importance that could cause serious outbreaks. Even though the current distribution of this species is quite limited in Europe, it has been theorised that it will spread all over Europe, and due to climate and global change it will thrive in urban environments (Wint et al., 2022).

Aedes albopictus is an extremely successful invasive species originally from Southeast Asia. This species has spread all over the globe due to the lucky bamboo (Scholte and Schaffner, 2008). It was detected for the first time in Europe in Albania in 1979, and then in Italy in the 1990s (ECDC, 2012). Since then, it has been detected in over 20 European countries including Spain (Figure 3) (ECDC, 2023). In Europe, its spread has also been facilitated due to ground transportation as it has been proven that this species travels inside vehicles (Eritja et al., 2017). *Aedes albopictus* was first detected in Spain in Catalonia in the year 2004 after citizen complaints of biting nuisance. Since then, other introductions have been reported in other regions of Spain (Collantes et al., 2015), including the Basque Country. This species has remarkably adapted to urban environments by breeding and developing in artificial containers (Bonizzoni et al., 2013). It is an aggressive and opportunistic biter and even though it feeds on a wide range of hosts, it has a preference to feed on humans (Giunti et al., 2023a). Due to its drought-resistant eggs, it overwinters thus surviving the winter season in temperate climates (Lounibos et al., 2003). These traits have likely facilitated the dispersal of this invasive species (Bonizzoni et al., 2013).

Aedes albopictus is another competent species that can transmit several pathogens such as the virus of dengue, chikungunya and Zika (Giunti et al., 2023a). In fact, since its establishment in Europe, there have been several autochthonous outbreaks that have been linked to this species (Aranda et al., 2018; Giunti et al., 2023a).

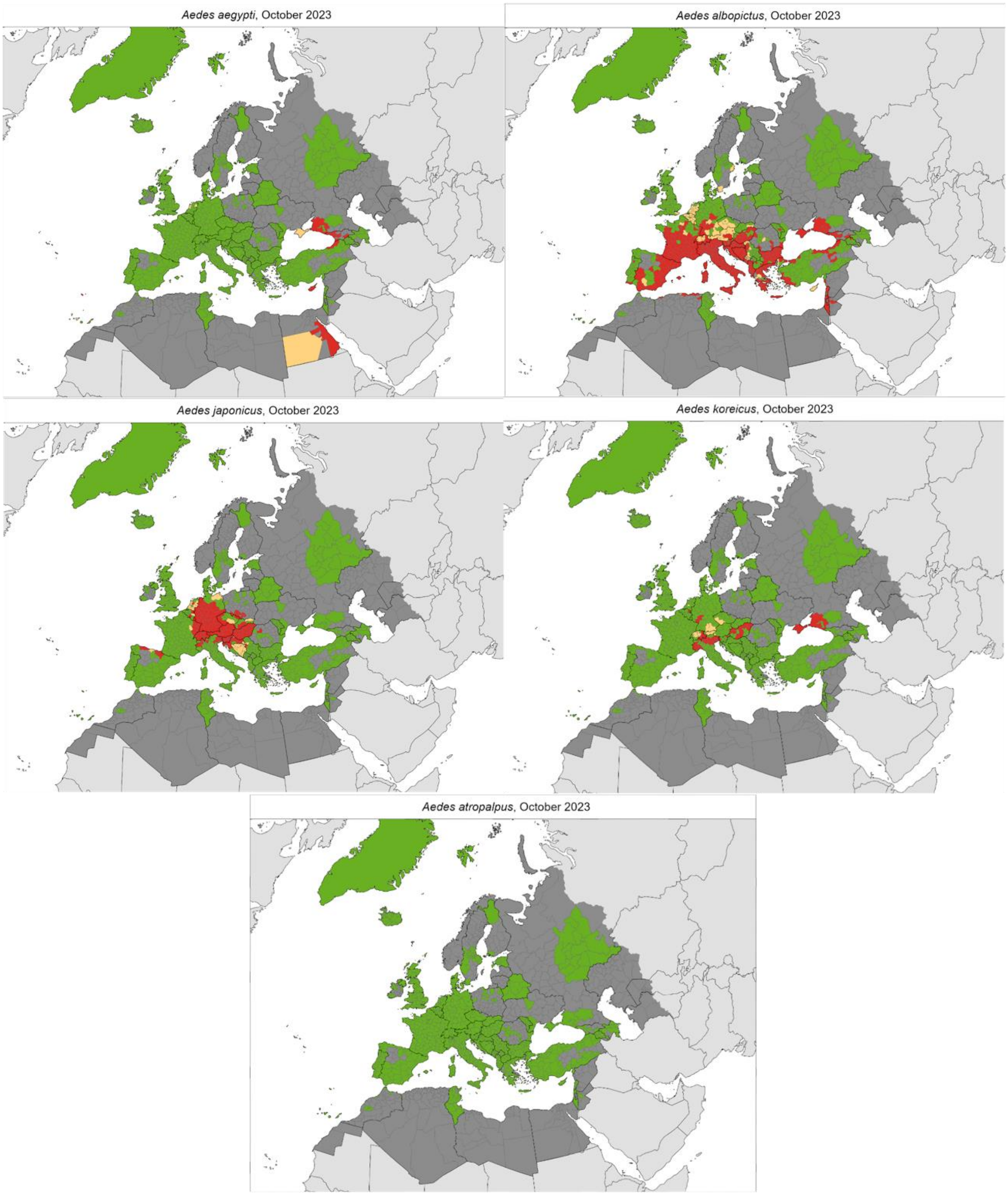

Figure 3. Distribution maps of *Aedes* invasive mosquitoes (ECDC, October 2023) (the map of *Ae. triseriatus* is not available).

Aedes japonicus is an Asian mosquito originally from Japan, Korea, south of China, Taiwan, and the eastern area of the Russian Federation (Tanaka et al., 1979). The reasons for this mosquito species to become invasive are still unclear, however, it is assumed that their drought-resistant eggs have been introduced through the same routes and trade transportation that *Ae. albopictus* (Kampen and Werner, 2014). It was detected for the first time outside its native range in New Zealand in the 90's in shipments with used tyres, along with *Ae. albopictus* (Laird et al., 1994). In Europe, it was detected in France in the year 2000, in a storage yard of imported used tyres. This first detection was eradicated using control measures (Schaffner et al., 2003). Since then, it has been detected in Germany (Werner et al., 2012), Switzerland (Schaffner et al., 2009), and Belgium (Versteirt et al., 2009), among other countries (Kampen and Werner, 2014; Koban et al., 2019; Montarsi et al., 2019) (**Figure 3**). In Spain, it was detected in Asturias in 2018 through in the citizen science platform Mosquito Alert (Eritja et al., 2019). Moreover, in 2020 it was also detected in the autonomous region of Cantabria and in the Basque Country (Eritja et al., 2021). Adults are active in the early spring and in the late fall. *Aedes japonicus* lays eggs in natural and artificial containers (Kaufman and Fonseca, 2014). However, they prefer larger volumes of water in comparison to other *Aedes* invasive species, such as *Ae. albopictus* (Werner et al., 2012). This species is not an aggressive human biter (Kaufman and Fonseca, 2014) if compared with the above-mentioned invasive *Aedes*.

Moreover, *Ae. japonicus* has been identified as a vector of several arboviruses like the Japanese Encephalitis virus (Huber et al., 2014) and the West Nile virus (WNV) (Kampen and Werner, 2014; Wagner et al., 2018). It has also been reported as a potential vector of St. Louis encephalitis, eastern equine encephalitis, La Crosse virus, dengue, and chikungunya (Kampen and Werner, 2014).

Aedes koreicus is closely related to *Ae. japonicus* as they belong to the same monophyletic group. In fact, they lived in sympatry and have been confused for a long time (Ganassi et al., 2022). It is originally from Japan, China, Korea, and eastern Russia. In 2008, it was detected for the first time outside its native range in Belgium and since then it has been successfully established (Versteirt et al., 2014). In Europe, it has also been detected in Italy (Capelli et al., 2011), Germany (Werner et al., 2016), Switzerland (Suter et al., 2015), Hungary (Kurucz et al., 2016) and Slovenia (Kalan et al., 2017), among others (Figure 3). It is a species well adapted to colder climates as its eggs are resistant to cold and drought conditions, and the adults survive until the autumn season (Ganassi et al., 2022). Moreover, due to this adaptation to colder climates, it can colonize areas with harsh winter temperatures, thus avoiding competition with other species, such as *Ae. albopictus* (Baldacchino et al., 2017).

As well as the other IMS mentioned, *Ae. koreicus* is also a potential vector of several pathogens such as Japanese encephalitis virus (Shestakov and Mikheeva, 1966), chikungunya virus (Ciocchetta et al., 2018), and the microfilariae dog heartworm *Dirofilaria immitis* (Montarsi et al., 2014). However, the competence of this vector is still under debate and further studies are needed (Capelli et al., 2011).

Aedes atropalpus also known as the American rock pool mosquito, has recently been reported in Europe. It is originally from North America and has spread to new territories due to commercial transport of used tyres (Nawrocki and Craig, 1989). It has been reported in France (Medlock et al., 2015), Italy (Romi et al., 1997) and in the Netherlands (Scholte et al., 2009). Although, it seems all these introductions have been controlled and eliminated (Medlock et al., 2015) (Figure 3). It is an autogenous mosquito, and larvae can be found in water-filled cavities of rocks (Burkett-Cadena, 2013). However, as it was mentioned earlier, it has adapted to use human-made containers and used tyres as oviposition sites (Yee, 2008). Like the IMS mentioned above, it is a multivoltine species that prefers feeding on mammalian hosts, including humans (Medlock et al., 2015). It has been described as an annoying biter in aquatic environments during the day. It is a competent vector of La Crosse virus (Freier and Beier, 1984) and WNV (Turell et al., 2001), although its importance as a vector of infectious diseases is still under debate (Scholte et al., 2009).

Aedes triseriatus is a North American invasive mosquito species that is widespread in the United States. Although it has only been reported in Europe once, it was intercepted in a batch of used tyres imported from Louisiana (USA) to France in 2004 (Giunti et al., 2023b). Its ability to overwinter as diapausing eggs and utilise artificial container habitats as larval development sites indicates that there is a risk that this species will establish in Europe (ECDC, 2023a). It is the primary vector of La Crosse virus, which has caused serious disease in humans in North America (Borucki et al., 2002) and has been suggested as a possible bridge vector for WNV, with field-collected adult mosquitoes testing positive for the virus. It has also been shown to transmit several other important human arboviruses under laboratory conditions.

These IMS have most likely been introduced due to transport of goods and international travelling. In fact, the trade of used tyres, together with the import of lucky bamboo are considered the main routes of entry of these IMS (Medlock et al., 2015; Schaffner et al., 2013). However, passive transport via ships, airplanes and cars are also involved in the dispersal of these species (Ibáñez-Justicia, 2020).

I.1.5.2. Native mosquitoes

In Europe there are 11 different biogeographic regions in where different habitats and species live. In fact, the biodiversity of natural species in Europe is low compared to the rest of the world (Condé et al., 2002). In the European continent, Northern Africa, South Caucasus, and part of the Middle East, there are 147 mosquito species (Robert et al., 2019). A latitudinal biodiversity gradient with the increase of the species richness from the poles to the tropics is observed in culicid species (Foley et al., 2007). This pattern is also observed in the different countries of Europe, as the species richness from Nordic to Mediterranean countries increases. In fact, in Nordic countries, 36 different mosquito species have been reported in Norway (Robert et al., 2019), 43 in Finland (Culverwell et al., 2021), and 51 in Sweden (Lindström and Lilja, 2018; Lundström et al., 2013; Robert et al., 2019). While in Central Europe, 47 species have been recorded in Poland (Kubica-Biernat, 1999), 53 in Germany (Kampen et al., 2017, 2013) and 53 in Hungary (Sáringer-Kenyeres et al., 2018). In western Europe, 38 culicid species have been identified in the Netherlands (Ibañez-Justicia et al., 2015; Scholte et al., 2010), 36 in the British Islands (Harbach et al., 2017), 60 in continental France (Robert et al., 2019). In southern Europe, 56 species in Greece (Samanidou-Voyadjoglou and Harbach, 2001), 63 in the continental Italy (Severini et al., 2009) and 43 in continental Portugal (Gouveia de Almeida, 2011; Robert et al., 2019). Regarding Spain, 66 mosquito species have been reported, belonging to seven genera: *Anopheles*, *Aedes*, *Coquillettidia*, *Culex*, *Orthopodomyia* and *Uranotaenia*, including exotic invasive and native species, uncertain species (*Anopheles superpictus*; *Anopheles labranchiae; Ae. aegypti* – in Canary Islands), and already extinct species (*Aedes dorsalis*) (Eritja et al., 2019; Robert et al., 2019).

The distribution maps of some native mosquitoes in Europe (*Cx. pipiens* group*, Culex modestus, Coquillettidia richiardii, An. superpictus, Anopheles plumbeus, Anopheles maculipennis* s.l.*, Ae. vexans, Ae. detritus/Aedes coluzii*, and *Ae. caspius*) are available in: https://www.ecdc.europa.eu/en/disease-vectors/surveillance-and-disease-data/mosquito-maps (ECDC, 2023b).

The *Culex pipiens* group is composed of two species: *Cx. pipiens* s.l. and *Cx. quinquefasciatus*. They are known as the "northern and southern house mosquito", being ubiquitous in temperate and tropical regions respectively (Fonseca et al., 2004). *Culex pipiens* s.l. is the most common species in Europe (Brugman et al., 2018). It has three different ecological ecoforms: *Cx. pipiens molestus*, *Cx. pipiens pipiens,* and their hybrids (Becker et al., 2012). The *molestus* form does not diapause and thrives in belowground habitats like basements and subways. The *pipiens* form is an aboveground mosquito that bites primarily birds, while the *molestus* form tends to be anthropophilic (Haba and McBride, 2022). Another key difference between these ecoforms, is that the *molestus* ecoform is autogenous, meaning they can lay the first batch of eggs without needing a blood-meal, while the *pipiens* ecoform does not have this ability (Haba and McBride, 2022). Besides, *Cx. pipiens* s.l. is an opportunistic species, feeding on the first available host, thus posing a major threat to the human population, as it is a known vector of the WNV (Fonseca et al., 2004; Soto et al., 2023).

Moreover, *Culex torrentium* is a sibling species of the *Cx. pipiens* complex. Females of these two species are very difficult to morphologically differentiate, and consequently it is difficult to know its distribution. In fact, initially it was considered a rare European species, however, currently *Cx. torrentium* is a widespread species in northern and central regions of Europe. Thus, in northern regions of Europe *Cx. torrentium* dominates, while in central Europe both species, *Cx. pipiens* and *Cx. torrentium*, have similar proportions. However, in southern regions of Europe the dominant species is *Cx. pipiens* and *Cx. torrentium* is rarely reported (Hesson et al., 2014).

Culex modestus is a wide distributed mosquito in the southern and central European countries (Balenghien et al., 2006; Becker et al., 2020). However, its distribution is seemingly expanding to other areas of the European continent (Hernández-Triana et al., 2020), being found established in England (Golding et al., 2012), Denmark (Bødker et al., 2014) and Sweden (Lindström and Lilja, 2018). It is a multivoltine and annoying biter mosquito and can be commonly found in marshes, coastal areas, rice fields and irrigation canals (Hawkes et al., 2020). This species can tolerate fresh to slightly brackish water (Hawkes et al., 2020). As for its host preferences, it is an ornithophilic species, but it also feeds on horses and humans (Balenghien et al., 2006). In fact, this species is a main vector of WNV in Europe and likely vector for Usutu virus, avian malaria (*Plasmodium* spp.), and parasitic heartworms (*Dirofilaria* spp.) (Soto and Delang, 2023). Due to its ornithophilic and mammophilic behaviour, it also acts as a bridge in the transmission of WNV between birds and humans (Balenghien et al., 2006). In addition, it has also been associated in the transmission of other infections, like Tahyna virus in humans (Danielova and Holubova, 1977). Moreover, it has been reported as a vector of Sindbis and Lednice viruses (Lundström, 1994).

Culex perexiguus is another important species, due to its vectorial capacity. It is a common species in Southwest Asia towards India and Northern Africa (Becker et al., 2020; Harbach, 1988), but it also can be found in Greece (Samanidou-Voyadjoglou and Harbach, 2001), Italy, Sicily, Malta (Gatt, 2009) and the Iberian Peninsula (Bueno-Marí et al., 2012). This species is closely related to *Culex univittatus* and have been misidentified in the past. However, Harbach (1999) clarified that the previous records of *Cx. univittatus* in southern Europe should be regarded as *Cx. perexiguus*. Despite this, both species are present in Spain (Bueno-Marí et al., 2012; Mixão et al., 2016). Even though it is considered mainly an ornithophilic species (Muñoz et al., 2012), it has been found fed on humans (Osório et al., 2012). Moreover, *Cx. perexiguus* is an important vector of WNV, associated with the outbreak in 2020 in Andalusia (Figuerola et al., 2022).

There are around 20 different *Anopheles* species in Europe, the most important from an epidemiological point of view is the widely distributed *An. maculipennis* complex (Robert et al., 2019). This complex is important in public health as it is a vector of malaria. In fact, it was the vector involved in the transmission of this disease in Europe until mid-20th century (Bruce-Chwatt & de Zulueta, 1980). Currently, this complex is composed of 11 species in the Palearctic region (Kampen et al., 2016b). In Spain, four species have been described, including *An. atroparvus*, *An. maculipennis* s.s, *An. melanoon* and *An. labranchiae*. However, the latter seems to have disappeared since 1973 (Bueno-Marí et al., 2012). Regarding *An. atroparvus*, it is considered the main vector of human malaria in Spain in the past. Moreover, it has been identified as the vector involved in the first malaria autochthonous case in 2010 since its eradication in 1964 (Santa-Olalla Peralta et al., 2010). *Anopheles atroparvus* distribution ranges from Portugal along the coasts of the Altantic up to south-eastern Sweden, and around the Baltic and Mediterranean Seas (Becker et al., 2020). It is an opportunistic species, feeding on the first available host, it prefers domestic animals, but will also readily bite humans (Danabalan et al., 2014; Martínez De La Puente et al., 2013).. A recent work reports the distribution of *An. maculipennis* s.l. in Spain (Taheri et al., 2024). The 78,6% of the total individuals analyzed were *An. atroparvus*. The remaining 21,4% were identified as *An. maculipennis* s.s., mainly found in northern Spain. Thus, *An. atroparvus* is the most common species within the *An. maculipennis* complex in Spain.

Another interesting *Anopheles* species is *An. plumbeus.* It is a Palearctic species widely distributed throughout Europe, although it can also be found in North Africa, the Caucasus, and the Middle East (Becker et al., 2020; ECDC, 2023b). The female is an annoying biter with a preference for mammal hosts, including humans (Heym et al., 2017), but have been found also feeding on birds and reptiles (Service, 1971). Moreover, some populations have shown a strong anthropophilic behaviour (Dekoninck et al., 2011; Schaffner et al., 2012). It usually breeds in tree holes with decomposing organic matter where they lay their eggs (ECDC, 2023b), but occasionally it has been found in artificial containers, rock, or ground depressions with fallen leaves (Aitken, 1954). It is usually found in forests and natural areas, but it can also be found in urban areas, like parks (Becker et al., 2020). In fact, a change in their habitat preference towards human environments has been observed (Dekoninck et al., 2011) and as well, its distribution and abundance has increased in recent decades in Central Europe (Dekoninck et al., 2011;

Schaffner et al., 2012). As an anthropophilic mosquito it can cause nuisance in the population (Heym et al., 2017) and could act as a bridge vector of malaria (Schaffner et al., 2012), and arbovirus (Van den Eynde et al., 2023).

Coquillettidia richiardii is also a common species in Europe and widely distributed in the western Paleartic region. It is a univoltine species having only one generation per year. When mosquitoes hatch, they are very numerous and are persistent biters to humans and domestic animals (Brugman et al., 2017a). Larvae are very difficult to find, as they are specialised in obtaining oxygen from specific aquatic plants. It can be found in freshwaters or slightly saline marshes, lakes, riverbeds, and estuaries (Becker et al., 2020). Several Trypanosomatids were found parasitizing *Cq. richiardii* (three *Trypanosoma*, three *Crithidia* and one *Herpetomonas* species) in Austria (Schoener et al., 2018). *Dirofilaria* spp. were also identified in this species (Tomazatos et al., 2018). However, its feeding behaviour raises some concerns as they feed both in birds and humans meaning a possible arbovirus transmission between species. Moreover, it is frequently found feeding indoors but usually the nuisance they cause is restricted to the surrounding areas of their breeding sites (Hawkes et al., 2020).

Another important Culicidae genus is *Culiseta.* The species belonging to this genus are dark and medium to large size mosquitoes (Becker et al., 2020). *Culiseta annulata* is an important species due to its abundance and nuisance behaviour (Hawkes et al., 2020). It is a Palearctic species, with a wide distribution around Europe, but it is more common in the North than in the South (ECDC, 2023b; Hawkes et al., 2020). It is an anthropophilic and aggressive human biter, but also bites other hosts like birds and other mammals such as rabbits, pigs, and other livestock animals (Hawkes et al., 2020). This species breeds in a wide range of aquatic habitats and can tolerate brackish water (Becker et al., 2020). They lay their eggs in rafts, usually around 200, and they can have several generations per year (Becker et al., 2020). Moreover, they do not diapause, as they are able to survive the winter season in all stages, with the adults hibernating in attics, cellars, and animal shelters (Becker et al., 2020; Hawkes et al., 2020). Its vector status is still unclear; however, it has been considered a potential bridge vector of WNV and Usutu virus (Martinet et al., 2019).

Another abundant *Culiseta* species is *Culiseta longiareolata*, which is widely distributed in the Mediterranean region, such as in Spain and Portugal. It can also be found in the east of Turkey, France, Switzerland, and Southern England (Becker et al., 2020). However, its distribution range is expanding to northern European countries, having been found in Germany (Kampen et al., 2013), Austria (Zittra et al., 2014), Slovenia (Seidel et al., 2013), Belgium, and the

Netherlands (Deblauwe et al., 2021). This expansion could be due to the warmer temperatures registered in Europe and to landscape anthropization (Deblauwe et al., 2021). It is an ornithophilic species that rarely bites humans (Becker et al., 2020). It breeds in rock pools and artificial containers such as wooden and metal barrels (Becker and Hoffmann, 2011). Moreover, the immature stages can tolerate high degree of pollution (Becker and Hoffmann, 2011) and slightly saline water (Becker et al., 2020). *Culiseta longiareolata* larvae are filter feeders but are also predatory, and even exhibit cannibalism behaviour (Becker and Hoffmann, 2011). This species lay eggs rafts, usually in the water surface (Becker and Hoffmann, 2011). Besides, *Cs. longiareolata* has been occasionally found in used tyres of import companies (Deblauwe et al., 2021; Roiz et al., 2007), being another possible explanation for its expansion throughout Europe (Becker and Hoffmann, 2011; Deblauwe et al., 2021). Due to its ornithophilic feeding behaviour, the species might be a potential vector of bird pathogens, such as WNV and avian malaria (Deblauwe et al., 2021), however its vector role has been poorly studied (Deblauwe et al., 2021; Martinet et al., 2019). Moreover, it has also been considered as a potential vector of Usutu virus (Seidel et al., 2013).

Among the native species in Europe of the genus *Aedes* the most relevant from a health point of view are *Aedes cantans, Ae. caspius, Aedes communis, Aedes cretinus, Ae. geniculatus, Aedes pulcritarsis, Aedes vexans* and *Aedes zammitii.* However, some of these are not present in Spain (*Ae. communis* and *Ae. zammitii*) (Robert et al., 2019).

Aedes cantans is a widespread univoltine species with a western Palearctic distribution (Becker et al., 2020; ECDC, 2023b). It is easily confused with *Aedes annulipes* as they are included in the same *Aedes* group of species (ECDC, 2023b). *Aedes cantans* lay their eggs in damp leaves of meadow pools, in shaded woodlands subject to flooding (Hawkes et al., 2020; Medlock and Vaux, 2015b). They share breeding habitat and occur together with other *Aedes* species like *Ae. annulipe*s, *Ae. communis* and *Ae. punctor* (Becker et al., 2020). Population density is at its highest during the summer (Hawkes et al., 2020). Its feeding host preferences include cattle, sheep, horses, birds, rabbits, and humans (Börstler et al., 2016), being an aggressive human biter (Hawkes et al., 2020). Due to their feeding preferences, biting humans and birds, it can act as a potential bridge vector of arboviruses. In fact, it has tested positive for WNV (Hubálek and Halouzka, 1999); however, its vector competence is still unknown (Martinet et al., 2021).

Aedes geniculatus is a common species in most European countries (Becker et al., 2020). It is easily confused with its sibling *species Aedes equinus*, and as well as with *Aedes* spp. invasive species like *Ae. triseriatus* (ECDC, 2023b). It is a tree hole breeding mosquito, but also can lay its eggs in artificial containers (Prudhomme et al., 2019). Adults are usually found in deciduous or mixed forest (Becker et al., 2020; ECDC, 2023b), during the summer. Females are aggressive biters, feeding on various mammals including humans, but also on birds and reptiles (ECDC, 2023b). Usually feeding takes place during the day or twilight hours, and in forested areas it can be a nuisance biter for humans (ECDC, 2023b). However, this species has also been reported in peri-urban areas (Yates, 1979). Its role as vector of diseases is still poorly investigated (Martinet et al., 2019). However, *Ae. geniculatus* has been proven in the laboratory to be a competent vector of chikungunya virus (Prudhomme et al., 2019), Sindbis virus (Jansen et al., 2022), and an efficient vector for *D. immitis* and *Dirofilaria repens* (Silaghi et al., 2017).

Aedes cretinus and Ae. pulcritarsis are easily confused with invasive species in Europe (ECDC, 2023b). Therefore, these species should also be taken into consideration when identifying mosquito species.

On the other hand, *Ae. vexans* is a widely distributed mosquito in Europe and can be found in nearly every country of this continent. It is a multivoltine mosquito and usually breeds in areas that get flooded like floodwater rivers, or lakes with water fluctuations. Moreover, it can get very abundant during the summer months and feeds both in human and domestic animals (Reinert, 1973). In Central Europe it hatches during the springtime after the flooding of the soil, however not all eggs hatch after the first flooding, needing to dry out and then flooding again. It is considered a "summer species" as its optimum temperature for their development is 30ºC, and due to becoming a dominant species and persistent biter during these summer months in temperate regions (Becker et al., 2020). *Aedes vexans* is considered an ideal vector species as it is widely distributed, can become very abundant, it feeds on humans and domestic animals and is present at the same time when the activity of viruses is high (Reinert, 1973). In fact, it has been discovered naturally infected with WNV in Serbia (Petrić et al., 2017).

Aedes caspius is also an aggressive human biter with a wide distribution in Europe (Robert et al., 2019). It is a multivoltine species and larval stages can stand and tolerate fresh and brackish waters (Hawkes et al., 2020). It is a typical mosquito in coastal marshes and flooded areas around estuaries, as well as in Atlantic and Mediterranean coastal marshes and rock holes. Even though it is considered an exophagic species, it has been reported entering dwellings. It is also a species with tolerance to heat and drought. The first adults emerge around April and through the summer until September, when their abundance increases (Milankov et al., 2009). In natural populations of *Ae. caspius,* WNV (Martinet et al., 2019), Tahyna virus and the bacteria *Francisella tularensis* (etiological agent of tularemia), have been detected (Detinova and

Smelova, 1973). Besides, in the laboratory it has been proven to be a competent vector of WNV (Balenghien et al., 2008; Martinet et al., 2019), and it has been considered the main vector of Rift Valley fever virus in Egypt (Gad et al., 1999).

Aedes detritus is a multivoltine species known as the “salt marsh” mosquito, as it breeds in high concentrations of salt water. It is a Palearctic species and can be found in most of the European coastlines (Becker et al., 2020). It is also an aggressive and anthropophilic species that, together with *Ae. caspius,* causes nuisance in coastal areas in Europe (Medlock and Vaux, 2013). Moreover, in the laboratory or in field specimens has been proven to be a competent or potential vector of WNV, dengue, chikungunya or Usutu viruses (Blagrove et al., 2016; Martinet et al., 2019).

Finally, *Uranotaenia unguiculata* is the only species belonging to the *Uranotaenia* genus present in Europe (Giunti et al., 2023a). It is mostly present in the Mediterranean region and as far as Germany (Kurucz et al., 2017). *Uranotaenia unguiculata* is usually found in forested areas, laying its eggs in rafts in the water usually in pools, ditches, or canals of stagnant or low-flowing water (Becker et al., 2020). This species primarily feeds on amphibians and reptiles (Toma et al., 2014), but it also has been reported to feed on birds and mammals, including humans (Becker et al., 2020; Giunti et al., 2023a). Due that its preferred hosts are cold-blooded animals, its role in disease transmission is minimal (Giunti et al., 2023a). However, *U. unguiculata* has been found naturally infected by *D. repens* (Șuleșco et al., 2016) and WNV (Kemenesi et al., 2014; Pachler et al., 2014).

I.2. Factors associated with mosquito distribution and abundance

Over the course of a 100-million-year mosquitoes have evolved and adapted to thrive in a wide variety of environments. They can be found all over the world, except in Antarctica and some islands (Mehlhorn, 2016). Water is an essential requirement for the development of mosquitoes (Wilkerson et al., 2021), and they can colonize temporal as well as permanent water, whether it is polluted or clean, even in larger or smaller waterbodies such as buckets, flowerpots, and leaf axes, among others. In fact, they are also able to adapt to different changes in climatic and environmental conditions. This immense ecological plasticity is the key to their success (Calzolari, 2016).

There are great variations in the bionomics in adult mosquitoes involving their host-seeking, biting and migration behaviour as well as their reproduction strategy (Clements, 1999). An essential factor for mosquito feeding is the availability of hosts. Depending on the species they either stay near their breeding sites, or they move actively searching for a host. For example, snow-melt mosquitoes, such as *Ae. rusticus*, stay around their breeding sites (Schäfer et al., 1997). However, other species such as *Ae. vexans* or *Aedes sticticus* have been trapped at distances of up to 11 km from their breeding sites (Brust, 1980).

Even though the number of mosquito-borne diseases (MBD) in Europe and the risk of human outbreaks is low comparing to tropical regions (Calzolari, 2016), there is an increasing trend in their global incidence as well as their geographical distribution. This is due to the increase of anthropogenic environmental changes, together with globalization that are creating the perfect conditions for the emergence and re-emergence of these diseases across Europe Haga clic o pulse aquí para escribir texto..

I.2.1. Climate change

Warmer climatic conditions favour the expansion of tropical species, such as *Aedes* invasive mosquitoes (AIM). It is estimated that by 2030 the yellow fever mosquito, *Ae. aegypti*, could find favourable conditions in the Iberian Peninsula (Iwamura et al., 2020). The climatic models for *Ae*. *albopictus* show that this specie has the fitting conditions in the Mediterranean regions in the South of Europe (Cunze et al., 2016). Moreover, it will find more suitable conditions as the climate change advances in Europe. Thus, the increase in the temperature could cause that *Ae. albopictus*, would be more active for a longer period throughout the year. Moreover, the rise in the temperatures would boost the life cycle of the mosquito, therefore being able to complete the cycle more times during the same year (Ryan et al., 2019). This could cause an exponential increase in the abundance of these insects, increasing problems in the well-being and health of citizens (Iwamura et al., 2020).

In contrast, *Aedes japonicus* has its suitable habitat in temperate regions of Central Europe, and as climate change progresses its distribution will decrease as it is not able to adapt to warmer climatic conditions (Cunze et al., 2016). On the other hand, a study carried out with *Ae*. *koreicus* showed that temperatures over 28ºC were not suitable for the development of the pupae and adults (Blagrove et al., 2016).

The effect of climate change, specifically in the increase of the temperatures, would affect three different aspects in the transmission of vector-borne diseases: the vector biting rate, vector development rate and pathogen replication. Therefore, the incubation period or the time needed for the vector to feed on a host and being able to transmit the pathogen would be shortened (Kilpatrick and Randolph, 2012). Consequently, MBD would be aggravated by climate change, as the contact between people and the pathogens will be facilitated (Mora et al., 2022) due to the expansion of the geographical range of these diseases and their vectors (Chen et al., 2011a). Besides, the warming in higher latitudes would allow the vectors and pathogens to survive the winter causing a higher exposure to these diseases along the year (Mora et al., 2022). Moreover, drought was also associated with the aggregation of mosquitoes and birds in the remaining water sources promoting the transmission of WNV (Epstein, 2005).

On the other hand, most of the studies about the expansion of mosquito and MBD have been focused on the role of the temperature change (Caminade et al., 2014; Jetten and Focks, 1997; Paz, 2015). These studies assume that the temperature is a robust predictor of the mosquito population dynamics without considering the possible associations and interactions this variable can have (Franklinos et al., 2019). Among these other factors that can influence the expansion of the vectors is the variation in the precipitations. There is a lot of debate around how the precipitation pattern will change in the future, however the main consensus is that the frequency of extreme precipitation will likely increase the formation of mosquito breeding sites (Knapp et al., 2015), or reduce them due to the periods of drought and extreme flooding. However, not only the precipitation will affect, as it will also depend on the preferences of the selection of the breeding habitat of the different mosquito species (Davis et al., 2017). Furthermore, other hydrological factors, such as soil type, vegetation and evapotranspiration rates, that control temporary water body developments may also affect, although these processes are very difficult to model (Day and Shaman, 2008).

In fact, climate change models have been widely used to try to understand the risk of MBD at different scale dimensions, their predictions have not always been accurate. At local scales they have been helpful to explain the variation of the incidence of some MBD (Siraj et al., 2014), but for larger spatial and temporal scales, analysing climate factors only, is not accurate. For example, it was predicted that, *Ae. aegypti* would occupy rural habitats of in the south of USA as it would have found suitable climatic conditions. However, these predictions turned out to be mistaken, as the competition of *Ae. albopictus* was not considered in the model (Lounibos and Juliano, 2018). In fact, *Ae. albopictus* has displaced *Ae. aegypti* in several states of USA (Hobbs et al., 1991; Lounibos et al., 2016) and in the Bermudas (Kaplan et al., 2010). However, in tropical cities like Bangkok (Stanton, 1920), Kuala Lumpur (Rudnick, 1965) and Calcutta (Gilotra et al., 1967), the opposite occurred where *Ae. aegypti* displaced *Ae. albopictus*. Nevertheless, climatic-based models of MBD should not be ignored if they consider multiple global change processes (Caminade et al., 2014).

I.2.2. Globalization

Other factors are also implicated affecting the geographical dispersion of these vectors, such as dispersal due to the movement of hosts, wind, trade routes, or biotic interactions, which include competition and predation (Peterson, 2008). Globalization has affected the distribution of many species of mosquitoes as they have been displaced and therefore colonized new areas (Meyerson and Mooney, 2007). However, this effect is not new, as the yellow fever mosquito, *Ae. aegypti*, was introduced in America during the 16th century through the trade of slaves (Powell and Tabachnick, 2013). However, the higher efficiency in the transportation has increased the movements therefore facilitating the colonization of new areas by IMS. In fact, global trade has increased in size, speed, and number of imports since 1970, and aviation as well has rapidly expanded, transporting billion of people each year (Hulme, 2009).

The problem with these alien species colonizing new areas is that they can alter the ecosystems as they can affect native species richness and abundance. In fact, they increase the risk of the extinction of native species and alter the trophic chain (Pyšek et al., 2020; van Riper et al., 1986). The main problem of the entry of these exotic mosquito species into new areas is the parallel introduction of new pathogens. For example, the introduction of the mosquito *Cx. quinquefasciatus* in the Hawaiian Islands caused a catastrophic effect on native bird species in the early 20th when they became infected by avian malaria, as malaria was not a disease present in these islands. Native species were more susceptible to this disease in comparison to introduced species (van Riper et al., 1986).

As already commented, mosquitoes can be transported by cars, trains, ships, and airplanes through long and short distances (Calzolari, 2016). The spread of *Ae. albopictus* all over the world is due to the trade of used tyres and plants. In fact, through this trade, the eggs of this species were transported and once the product arrives at its destination, due to their high plasticity they adapt and spread (Paupy et al., 2009). Moreover, it has been proven that mosquitoes can also travel inside cars, therefore being able to colonize new areas, as well (Eritja et al., 2017). In the recent decades the spread of IAM in Europe has been facilitated by ground transportation along the motorways (Ibáñez-Justicia, 2020). However, not only the vector is transported, as diseases are also imported (Kilpatrick et al., 2006) either directly by the introduction of an infected mosquito, either by human travelling or by the displacements of infected migratory birds, and wild or domestic animals (Calzolari, 2016). In fact, many diseases have been introduced into areas where the disease was either not present or eradicated, and as the vector of the pathogen was already present it has caused autochthonous outbreaks (Giunti et al., 2023). For example, infected mosquitoes by malaria have been transported by airplane from endemic countries and cases of airport malaria have been reported in Europe (Martens and Hall, 2000). Many vector-borne diseases have experienced an increase in their global incidence in the last decades (Kilpatrick and Randolph, 2012). This trend, together with the diseases and the outbreaks reported in Europe, will be mentioned in later sections.

I.2.3. Landscape anthropization

Another important factor modifying the distribution and abundance of mosquito species, as well as the incidence of pathogens that they transmit is the transformation of the landscape by humans. Land-use change is a process that has been taking place in the Anthropocene and is affecting the interactions between people, pathogens, vectors, and vertebrate hosts causing alterations in diseases risk (Gottdenker et al., 2014; Kilpatrick and Randolph, 2012). The Anthropocene is a proposed non-official new geological epoch, that is profoundly impacted by the changes made by human activity and greatly affects the environment (Lewis and Maslin, 2015), such as the urbanization (Gottdenker et al., 2014). Urbanization consists in the alteration of the natural environment to make the area more suitable for the accommodation and growth of the human population (Johnson and Munshi-South, 2017). In fact, due to the development and expansion of the cities, forested and rural areas surrounding them have been transformed into urban settings (Abdullah and Hezri, 2008). In 2018, 55% of the total population was reported living in urban areas, which is a substantial increase from the 34% accounted in 1960. This trend will likely increase in the future, as it has been projected that by 2050 2.5 billion people will reside in urban environments. In fact, 90% of this growth will be happening in Asia and Africa (United Nations, 2019). The increase in the population of urban settings could result in a rise of the risk of pathogen transmission for some MBD (Weaver and Reisen, 2010). It could increase the re-emergence of MBD, such as malaria, as it could enhance the interactions between vectors and hosts (Patz et al., 2004). Another example is the rise of dengue, chikungunya and Zika viruses, as urban expansion has influenced the availability of resources and climatic factors that alter mosquito community ecology. Urban areas experience warmer temperatures than rural areas surrounding them. This effect is known as “urban heat island”, and it can cause the reduction of time needed to complete the life cycle of the mosquito (LaDeau et al., 2015).

This increase in urbanization and landscape transformation could result in a loss of biodiversity (McKinney, 2002). Moreover, the impact of urbanization on the species richness varies with the geographic location and with the historical and economic factors of each city (McKinney, 2008). The alteration of the habitat can cause an increase in the abundance of a few species and the decrease of others (McKinney, 2008) as urbanization tends to eliminate the habitats of most native species, while invasive species are able to adapt and occupy these newly urbanised areas (McKinney, 2006). Therefore, depending on the mosquito species some are benefited by these anthropogenic changes, like for example AIM or *Cx. pipiens* s.l. (Wilke et al., 2021b). In fact, the simplification of the structure of the habitat and the alteration of the trophic interactions made by humans may be the cause of the lower biodiversity observed in urban settings (Ferraguti et al., 2022).

Furthermore, mosquito populations can be impacted by changes in the availability of resources, vegetation coverage and the characteristics of the water bodies, together with temperature and precipitations (Ferraguti et al., 2022). In fact, the rapid expansion of cities has aggravated the problem as environments surrounding them have been altered (Abdullah and Hezri, 2008). These changes in the landscape are providing suitable breeding habitats to mosquitoes (Ferraguti et al., 2022). Green urban areas, such as parks or roadside green, may end up being hotspots and support invasions of exotic mosquito species in cities (Becker et al., 2020; Ferraguti et al., 2022). As commented above, some AIM, like *Ae. albopictus* and *Ae. aegypti,* have adapted and breed and thrive in artificial containers, drains and gutters, abundant in the cities, with little competition or predation (Townroe and Callaghan, 2014). A recent study suggests that rapid urbanization together with climate change will result in a shift in the biting behaviour of *Ae. aegypti* turning it more anthropophilic in many large cities of sub-Saharan Africa by 2050 (Rose et al., 2020). Furthermore, Rose and collaborators (2020) suggest that *Ae. aegypti* may become dependent on humans because to survive during hot dry seasons, their only option may be laying eggs in human-made containers that retain water. Therefore, evolving and driving their preference to human biting.

The abundance and species richness of mosquitoes is generally lower in urban settings when compared to rural or environmental areas (Ferraguti et al., 2016). In fact, depending on the urban-rural gradient, mosquito diversity was different between these areas. For example, in urban and peri-urban areas from Peru, *Culex* mosquitoes were predominant, while in rural areas the genus *Mansonia* was the most common (Johnson et al., 2008). This is because *Culex* mosquitoes can breed and develop in a wider variety of habitats (Fischer and Schweigmann, 2004), including artificial breeding sites (Johnson et al., 2008). Moreover, the expansion of cities towards natural and rural environments could help in the establishment of exotic rural mosquitoes, such as *Ae. japonicus*, in this peri-urban areas (Bartlett-Healy et al., 2012; Kampen and Werner, 2014). This is because *Ae. japonicus* benefits in areas surrounded by small patches of forests (Ferraguti et al., 2022). In addition, habitat disruption and land cover affect vector-borne diseases, bringing them closer to the human population (Mora et al., 2022). This is the case of MBD such as Zika (Ali et al., 2017), yellow fever (Nava et al., 2017; Ribeiro and Antunes,

2009) and WN (LaDeau et al., 2011), among others. Besides, deforestation and alteration of rural environments are also important factors to be considered as these areas could be enzootic to diverse zoonosis, bringing closer vectors, pathogens, and hosts (Patz et al., 2004).

I.3. Medical and veterinary importance

More than half the population of the world is at risk of becoming infected by pathogens transmitted by mosquitoes. Moreover, the World Health Organisation (WHO) has estimated that every year there are more than 340 million cases and more than half a million deaths due to MBD (Franklinos et al., 2019; WHO, 2021). Even though most mosquito species live in tropical and subtropical regions, their risks still exist in temperate regions (Schaffner et al., 2013).

I.3.1. Biting nuisance

Mosquito biting nuisance derives from the females that require blood for the egg development. Bites can sometimes cause an itchy bump, as a response from the immune system of the host to the mosquito saliva. In the worst case, the bite can become infected by bacteria (Foster and Walker, 2019). The most well-known mosquito in temperate regions is *Cx. pipiens* form *molestus*, also called the “house mosquito”. This mosquito due to its widespread distribution as well as its presence in human settlements causes more complaints and has a bad reputation (Medlock et al., 2012a).

In certain situations, mosquito population is composed of anthropophilic species, meaning that they prefer feeding on humans. However, sometimes this preference may vary depending on the availability of other hosts (Takken and Verhulst, 2012). Public health nuisance complaints can be caused when the population of adult female mosquitoes is abundant and are intensively searching for hosts. This behaviour changes periodically through the seasons and their activity periods, and, depending on the flight range of the mosquito species if the human population is in proximity (Brugman et al., 2017b). Therefore, species with minor or no degree of anthropophilic preference even with high abundance, are not likely to cause problems among the human population (Hawkes et al., 2020). For example, *Cx. territans* primarily feeds on amphibians (Eubanks et al., 2008), and *Cs. longiareolata* is an ornithophilic species (Chordá Olmos, 2014).

Occasionally, the presence of mosquitoes and the perception of nuisance by the population does not match. For example, *Cx. pipiens* form *pipiens* is an ornithophilic species (Gomes et al., 2013). But, because during the cold months they need to shelter from cold temperatures, they are usually found inside the houses. This behaviour causes the impression of an unpleasantness in the community as they associate it with a biting risk (Medlock et al., 2012a). The same happens with the flying swarm's insect form, as they assume there is a possible biting risk even though it could possibly not be composed of any biting insect (Hawkes et al., 2020).

On the other hand, there are cases when a small population of anthropophilic mosquitoes can cause a great nuisance to the population. This occurs when these small populations are near human dwellings or when people visit areas near their breeding sites and habitats (Hawkes et al., 2020). The significance of the nuisance a mosquito can cause is determined mainly by its physiological characteristics which includes its reproduction, migration, host-seeking, and biting behaviour (Becker et al., 2020). For example, mosquitoes that live in water environments, such as marshlands, lagoons, rivers, or coasts, can cause great nuisance when their abundance increases. In fact, mosquitoes like *Ae. vexans* or *Ae. sticticus* emerge in great numbers and in addition, they have a flight capacity of several kilometres, thus becoming a nuisance even in distant locations from their breeding sites (Gjullin et al., 1950).

I.3.2. Host species preferences

Blood feeding preferences in mosquitoes have complex patterns as some species feed on a wide range of hosts. However, some species can be specialists and prefer to feed on a particular host or animal species, while others are generalists and feed on diverse animals being usually their host the first available species they encounter. Moreover, some species are opportunistic meaning that even though they may have a host preference, they will easily feed on a wide range of available hosts. The selection of the host in opportunistic mosquitoes will be probably determined by the most abundant species in the environment, or readily available (Takken and Verhulst, 2012). To classify mosquito species in a single category is difficult as it can depend on host availability in the environment (Fikrig and Harrington, 2021).

The wide range of host upon which mosquitoes feed can go from humans to frogs, including birds and small and big mammals. Depending on the specificity of the species they express this behaviour in different degrees (Takken and Verhulst, 2012). Some mosquitoes have a preference to feed on humans like *Ae. aegypti* and *Ae. albopictus*, among others (Reinhold et al., 2018). In fact, as commented above, in the *Cx. pipiens* complex feeding preferences vary between ecoforms, for example *Cx. pipiens pipiens* has been reportedly described mainly as ornithophilic, while his sibling ecoform *molestus* feeds on humans (Osório et al., 2014).

The selection of the host to feed on is crucial for the development of the life cycle of the pathogens. Some of these pathogens change the behaviour of the vector making them prefer a specific host. A study has reported that infected persons with Zika or dengue become more attractive for the mosquitoes, as they emit a chemical substance. This means that these kinds of viruses to get transmitted to another host they are able to alter people's odour to increase the possibility of being bitten by a mosquito (Zhang et al., 2022). This same situation had already been described with the Malaria parasite, *Plasmodium* spp. (Robinson et al., 2018).

There are some external factors that can affect the preference toward a particular host, being the olfactory sense the main detector of hosts in mosquitoes. In fact, odorants released by the host such as carbon dioxide is a common stimulus for all hematophagous arthropods. However, carbon dioxide indicates the presence of a host, as it is exhaled by all vertebrates, therefore it is not host specific (Takken and Verhulst, 2012). On the other hand, other skin emanations are host specific, such as lactic acid which is a product excreted by humans. In fact, lactic acid is an important cue in the selection of host in anthropophilic mosquitoes such as *Ae. aegypti* (Smallegange et al., 2011).

Moreover, it is also important the behaviour of mosquitoes (endophilic or exophilic) because it could increase the spread of MBD in humans. In fact, species like *Ae. aegypti* or *Ae. albopictus* are both described as endophagic mosquitoes, meaning they feed inside houses. *Ae. aegypti* is also an endophilic mosquito, seeking sheltered spaces to rest, whereas *Ae. albopictus* has been described as an exophilic species (Reinhold et al., 2018).

An interesting fact that has been observed, is that mosquitoes that are dehydrated are more aggressive and tend to feed more frequently than well hydrated mosquitoes. Moreover, this behaviour could be increased in drought periods thus causing a rise in the transmission of MBD (Hagan et al., 2018). In fact, it has been reported that during dry years, incidence of WNV increased, suggesting that climate alters the transmission patterns of vector-borne diseases (Paull et al., 2017).

I.3.3. Mosquito-borne diseases

Mosquitoes are vectors of many diseases as they can transmit pathogens including bacteria, viruses, protozoans, and nematodes. These pathogens cause important diseases like malaria, dengue, Zika, chikungunya, West Nile, yellow fever, Japanese encephalitis and filariasis, among others, threatening more than half of the world's population. These diseases have a big impact in the socio-economic development of countries in tropical regions, affecting tremendously the poorest populations (WHO, 2020a).

Even though they represent a lower risk in the temperate regions, MBD have also been present in Europe. In fact, both the vectors and pathogens are being introduced or reintroduced through international travel and trade, thus, concern is increasing in Europe. Several outbreaks have been reported all over Europe in recent years. Their emergence is usually related to changes in the ecosystems, human behaviour, and climate (Schaffner et al., 2013).

Following, in the next sections the most important diseases and their outbreaks in Europe will be reviewed to state the importance of mosquitoes in animal and human health.

I.3.3.1. Arboviruses

Arboviruses, also known as the arthropod-borne viruses, are viruses that replicate and are afterwards transmitted by arthropods to vertebrates. There are around 620 viruses described in arthropods, but only about 100 infect humans and 40 infect livestock. Four genera belonging to three families are involved in this transmission pattern: *Alphavirus* which belong to the Togaviridae family, *Orthobunyavirus* and *Phlebovirus* belonging to the Bunyaviridae family, and *Flavivirus* belonging to the Flaviviridae family (Foster and Walker, 2019).

Yellow fever (YF) is a haemorrhagic disease endemic in parts of Africa, South and Central America, caused by a *Flavivirus*. It is primarily transmitted by *Ae. aegypti*. The disease is called "yellow" because of jaundice that some patients develop. It was the first arbovirus identified as a mosquito-borne infection (Foster and Walker, 2019). Mortality can be as high as 75%. Even though an effective vaccine has been developed, there are still important outbreaks in unvaccinated populations. Moreover, it has been estimated that there are 200,000 cases of YF and 30,000 deaths every year, of which 90% occurring in Africa (CDC, 2018). It has three transmission pathways depending on where the mosquito species breed. The sylvatic YF takes place in the tropical rainforests, where the primary reservoir are the monkeys and where humans, either working or travelling, can be infected. Another pathway takes place in heavily populated urban centres where an abundant population of *Aedes aegypti* is present. In these areas, the virus is transmitted by the mosquito from person to person, and due to lack of vaccination or exposure to previous infections large epidemics can take place. In between these two pathways there is an intermediate that occurs when mosquitoes infect both humans and monkeys. This last one is the most common in Africa (WHO, 2019). Recent epidemics of YF have emerged in Angola and Congo in the year 2016 threatening even Asia, a region free of this disease but with the presence of its vectors. The outbreak was caused by infected travellers importing the viremia to China and due to presence of a wide *Ae. aegypti* population together with the unvaccinated population, this caused a major health threat to the Asian continent (Wasserman et al., 2016). Other outbreaks have also been reported in Brazil, reaching as well

YF-free areas, thus causing thousands of deaths both in humans and non-human primates (Silva et al., 2020).

Dengue is a disease caused by a *Flavivirus* transmitted by *Aedes* mosquitoes, primarily *Ae. aegypti*. It is the most prevalent viral infection with more than 3.9 billion people at risk of contracting it in 129 countries of the world. The WHO has estimated that every year there are 96 million clinical cases and 40,000 deaths. There are four dengue virus (DENV) serotypes (WHO, 2022a). The incidence of dengue has increased in the last decades due to the increment in international travelling. In fact, in Europe there has been several cases of imported dengue consequently leading to autochthonous outbreaks in several countries, such as in France (la Ruche et al., 2010; Succo et al., 2016), Spain (Monge et al., 2020), Italy (Lazzarini et al., 2020) and Croatia (Gjenero-Margan et al., 2011). These autochthonous outbreaks were due to the spread and establishment of the invasive *Ae. albopictus* (Gossner et al., 2022). In fact, in Barcelona the DENV was detected in local *Ae. albopictus* mosquitoes near the residence of a man after an international travel (imported case) (Aranda et al., 2018).

West Nile virus (WNV) is part to the Japanese encephalitis complex belonging to the Flaviviridae family. It has a wide expansion in Europe, the virus circulates in birds, with members of *Culex* spp. being likely the enzoonotic vectors. It has been theorised that migrating birds between Africa and Europe could be introducing and maintaining the circulation of the virus (Malkinson and Banet, 2002). The species of *Culex* that transmit WNV varies depending on the region (Ciota, 2017). For example, in Western U.S *Culex tarsaris* dominates the transmission (Goldberg et al., 2010), while in South Africa it is *Cx. univitattus* (Jupp, 2001), in Australia it is (Johnson et al., 2008)*Culex annulirostris* (Jansen et al., 2013) and in Europe it is *Cx. modestus* (Hubálek and Halouzka, 1999), *Cx. perexiguus* (García San Miguel Rodríguez-Alarcón et al., 2021), and *Cx. pipiens* (Rizzoli et al., 2015). Moreover, in the laboratory other European mosquitoes were tested on their vector competence for WNV and determined that, *Ae. albopictus*, *Ae. caspius*, *Ae. detritus, Cx. pipiens* and *Cx. modestus* were capable of transmission (Vogels et al., 2017a). In fact, the role of *Ae. japonicus* as a possible vector of WNV is still unresolved (Montarsi et al., 2019). Outbreaks of West Nile have been reported recently in Spain, with *Cx. perexiguus* acting as the main vector (García San Miguel Rodríguez-Alarcón et al., 2021). Human infections are usually asymptomatic and mild, however, a neuroinvasive disease (WNND) can be developed in immunocompromised and elderly people, which may lead to death (Gossner et al., 2017). Moreover, WNV can cause severe illness in horses, as encephalomyelitis can be developed. In fact, large epidemics have been reported in France (Murgue et al., 2001), Portugal (Barros et al., 2017) and Spain (García-Bocanegra et al., 2018). Infections usually take place during the summer months when mosquito populations are more abundant. There is no specific treatment nor vaccine available for humans, however inactivated and recombinant vaccines for horses are used in Europe (Gossner et al., 2017).

Zika is another *Flavivirus* that in recent years has gained great interest since the spread to new regions of the world. Infections are usually mild, however in pregnant women, the infection can cause abortions or malformations in the central nervous system of the foetus, sometimes causing microencephaly (Cauchemez et al., 2016; WHO, 2018). Moreover, the Zika virus can be also transmitted through sexual contact and from the mother to the foetus during pregnancy. *Aedes* mosquitoes are the vectors of this arbovirus, especially *Ae. aegypti* and *Ae. albopictus* (Chouin-Carneiro et al., 2020, 2016). Currently, there is no treatment or vaccine for this infection, therefore prevention lies on avoiding mosquito bites, especially among pregnant women (WHO, 2018).

An emerging mosquito-borne flavivirus with increasing prevalence in Europe is Usutu virus (USUV). USUV was initially isolated in 1959 in southern Africa and has also spread to Europe, primarily affecting birds and with a limited impact on human health. USUV has recently been gaining attention due to the diversity of its circulating lineages (Simonin, 2024). *Culex pipiens pipiens* is considered the primary vector of USUV.

Chikungunya virus (CHIK) is an *Alphavirus* that has caused many outbreaks in Africa, India, and south-eastern Asia in recent years. The main vectors of this disease are *Ae. aegypti* and *Ae. albopictus* (WHO, 2020b). The first important outbreak in Europe was reported in Italy in 2007 where more than 200 cases were confirmed (Rezza et al., 2007). Since then, several chikungunya outbreaks have been reported all over Europe like in France in 2010 (Grandadam et al., 2011), 2014 (Delisle et al., 2015), and 2017 (Calba et al., 2017), and in Italy again in 2017 (Venturi et al., 2017). Currently, there is no vaccine or specific drug against the virus, the only course of action is to avoid mosquito bites by eliminating potential breeding sites for *Ae. aegypti* and *Ae. albopictus*.

Rift Valley fever (RVF) is a disease caused by a *Phlebovirus*. It is a zoonotic disease endemic to sub-Saharan African countries that affects ruminants and humans. In ruminants, it causes abortions or foetal malformations. Sheep are the most susceptible to this infection (Ikegami and Makino, 2011). Humans can become infected either by a mosquito bite or more commonly if they are in close contact with infected animals, through their contaminated blood or by aerosols during slaughter, necropsy, and butchering, but no human-to-human transmission has been documented (Hartman, 2017). In humans the infection is rarely lethal, but it causes fever, headache, myalgia, retinitis and in extreme cases liver problems. Infection in animals causes huge economic losses (Foster and Walker, 2019). A wide range of mosquito species have been identified as possible vectors of this disease, including *Culex pipiens* s.l. and *Ae. vexans*, among other *Aedes* spp. species that breed in wetlands (Chevalier et al., 2010). There have been several outbreaks in Africa and the Middle East since its first description in 1930 (Ikegami and Makino, 2011). The impact of the disease in public health can be very severe. For example, in the outbreak in Egypt in 1976, it was reported that 200,000 people were infected and more than 600 died (Chevalier et al., 2010). However, due to trade and transport of livestock the disease could expand its range being its emergence a possibility in Europe. As well, climate change could also impact in the geographical distribution of this virus (Chevalier et al., 2010).

There is no specific treatment available yet for animals or humans. There is a vaccine available for humans, but the method recommended is the vaccination of the animals, as humans usually become infected after being in contact with the infected animals (Chevalier et al., 2010).

I.3.3.2. Parasites

Malaria is the most important vector-borne disease in the world affecting more than 100 countries, which places nearly half of the human population at risk. In 2020, the WHO estimated there were around 241 million cases of malaria, and 627,000 deaths. In fact, the number of cases has increased in comparison to previous years, due to the Covid-19 pandemic that has disrupted the prevention, diagnosis, and treatment services (WHO, 2021). This disease is caused by protozoans of the genus *Plasmodium* (*P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi*) (Kantele and Jokiranta, 2011). In fact, this protozooa has a complicated transmission cycle with a sexual replication in the mosquitoes and an asexual replication in the vertebrates.

During the first half of the twentieth century, malaria in Europe had a high impact in the population. It was a severe endemic illness, mostly in the Southern countries, but it also affected the Northern countries. Moreover, the two main *Plasmodium* species present in Europe were *P. vivax* and *P. falciparum*. In fact, *P. falciparum* was the parasite affecting the Southern parts of Europe, whereas *P. viva*x had a wider distribution throughout the continent (Bruce-Chwatt & de Zulueta, 1980). After World War II, the disease gradually started to disappear from the continent, as their natural breeding sites were reduced and because of the eradication campaigns. The reduction of the mosquito breeding habitats was a result of the drainage and canalisation of rivers, novel agricultural techniques and because of better sanitary practices and socioeconomic conditions. The eradication campaigns consisted of the application of insecticides helped with the development and availability of new medicines (Bruce-Chwatt & de Zulueta, 1980).

This disease is only transmitted by the anopheline mosquitoes. It seems that the *An. maculipennis* complex was the species spreading the disease in Europe as this species has a wide distribution in the Paleartic region. This complex has a higher vectorial capacity than other closely related species because of their anthropophilic behaviour and physiological viability. Although, other European anophelines also have the capacity to be vectors of this disease, like *An. claviger*, *Anopheles sergentii*, *Anopheles cinereus hispaniola*, *Anopheles algeriensis*, *An. superpictus* and *An. plumbeus* (Piperaki and Daikos, 2016). In fact, the last one has gained a lot of interest as in recent decades this mosquito has proliferated and can be found in high numbers. This could be due to their adaptation from natural breeding sites (tree holes) to artificial breeding sites like catch basins (Schaffner et al., 2012)

Filariasis is an infection caused by filarial nematodes belonging to the family Filariodidea that affects vertebrates. It is a widespread infection in tropical and subtropical regions, with 863 million people in 47 countries at risk. Moreover, it affected 51 million people in 2018. There are three species that cause the disease in humans: *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. The former is the one responsible for the 90% of the cases in the world (WHO, 2022b). The infection begins when a third-stage larvae enter the skin through a mosquito bite. Then, they migrate to the lymphatic vessels and lymph nodes where they develop to the adult stage. From the lymphatic vessels, the immature worms called microfilariae release into the peripheral circulatory system. This disease is transmitted by different types of mosquitoes like *Culex*, *Anopheles* or *Aedes* (Simonsen and Mwakitalu, 2013). In fact, depending on the region different species of mosquitoes are involved in the transmission cycle. The disease in humans is not life-threatening, however, it causes permanent damage in the lymph system and kidneys. Besides, when the illness develops into more chronic conditions, then it results in tissue swelling and thickening of limbs. The swelling is called lymphoedema, while the thickening of the skin is called elephantiasis (Foster and Walker, 2019). The treatment to stop the spread of this parasite is through preventive chemotherapy. In fact, more than 8.6 billion treatments have been dispatched since the year 2000 to eliminate the infection (WHO, 2022b). However, filariasis caused by *Dirofilaria immitis* in dogs is a more severe disease. After getting infected by a mosquito, the larvae migrate into their right ventricle and pulmonary arteries. This infection is called “dog heartworm” and can cause the death if not treated. The mosquito genera that can transmit this infection in dogs differs with the geographic region, as seen with the human disease (Foster and Walker, 2019). In Spain, the vector of this disease is still under debate, but it seems that the *Culex* genera is involved, as *Cx. pipiens* s.l. and *Cx. theileri* have been found naturally infected by *D. immitis* (Morchón et al., 2011, 2007).

I.4. Mosquito research techniques

I.4.1. Surveillance and field sampling methods

The mosquito field sampling methods are used to gain knowledge on the distribution, abundance, seasonality, and ecology of different mosquito species. Also, it can be used for the monitoring and surveillance campaigns of native and invasive species, thus these methods of capture can be classified depending on the objectives. Depending on the target species, the sampling sites selected as well as the sampling techniques, are different. Field sampling methodology can be divided into collection of pre-imaginal stages (eggs, larvae, pupae), flying and resting adults (Becker et al., 2020; ECDC, 2018).

I.4.1.1. Sampling mosquito eggs

Depending the different genera, mosquitoes lay their eggs either individually or in rafts, as well as in different habitats (e.g., water surface, wet ground, tree holes, man-made containers, rock pools) therefore different techniques must be employed depending on the target species and the available habitats in the sampling site selected (Becker et al., 2020; ECDC, 2018).

Anopheline mosquitoes lay their eggs individually on the water surface, and due to having structures like floats on each side, they float on the water. These eggs are difficult to see with the naked eye and they are collected using a light-coloured dipper. Also, a modification in the dipper adding a fine wire net has been employed. Individual eggs can be collected after several sweeps with the dipper upside down on the water. After, the net is washed in a light-coloured dish, where the eggs can be picked out using fine forceps or a pipette (Becker et al., 2020; Silver, 2008).

Other mosquito genera (e.g., *Culex*, *Coquillettidia*, *Culiseta*, etc.) lay their eggs in rafts on the water surface. These structures can be seen easily and therefore can be collected using fine forceps, small nets, or pipettes (Becker et al., 2020).

Egg sampling is especially useful for *Aedes* species, as this genus lays their eggs individually into a substrate just above the water level. The eggs are extremely small, and thus seeing them is especially difficult. Each *Aedes* species prefers specific locations for depositing their eggs.

Some *Aedes* females like to deposit their eggs in wet soil, therefore soil samples from the breeding sites must be collected (Becker et al., 2020)

Other *Aedes* species, especially AIM, deposit their eggs on the inside of small containers just above the water level. Therefore, the use of small artificial containers, called ovitraps, is a good choice for the surveillance of AIM, such as *Ae. albopictus*, *Ae. aegypti*, *Ae. japonicus*, among others. Ovitraps consist of a water holding container and a substrate, such as masonite, where females lay individually their eggs. Ovitraps are usually deployed in wind protected and shaded sites. Dechlorinated tap water is added, and the water level is controlled with a hole in the plastic container to prevent overflow. The substrates are changed at a regular interval (7 to 15 days) and transported to the laboratory for examination under a stereomicroscope where eggs are counted. This method is cheap, but identification of the species is time-consuming, because includes the hatching of larvae and the moult to adult mosquitoes. Moreover, a direct correlation between the number of eggs deposited and the female density cannot be made, as females deposit their eggs in various locations (Becker et al., 2020; ECDC, 2014).

I.4.1.2. Sampling mosquito larvae and pupae

Larvae and pupae are the aquatic stages of mosquitoes, collecting these stages allows a rapid inspection and identification of the species present in an area. These immature forms inhabit in an extensive range of environments depending on the species and the sampling area, different locations must be inspected. For instance, six different types of larval habitats can be classified: man-made artificial water containers, natural containers (e.g., tree holes, rock pools), permanent water bodies with vegetation, natural water bodies without vegetation, stagnant temporary water bodies and running waters (ECDC, 2018).

Based on the type of larval habitats and their size, these stages can be collected either with a dipper, a net or aspirating the water. Normally dipping is the technique used, however, as the dippers can differ in size and capacity, it is useful to measure the volume capacity of the tool used, as a mean to standardise the method. To estimate the number of immature stages, present in the habitat, the capacity of the dipper, the number of dips, and of larvae and pupae must be recorded. Moreover, it is also useful that the dipper has a light colour to discern the larvae. This method is very effective for sampling large bodies of water. As for smaller water bodies, such as tree holes or rock pools, aspirating the water with a pipette is more practical. Inmature stages of *Coquillettidia* spp. are not easily detected using standard dipping methodologies (Medlock and Vaux, 2015b) because they are fixed to plant tissues underwater in order to use the oxygen from aquatic plants (i.e. *Typha* sp.), aerenchyma (Bosak and Crans, 2002). Thus, aquatic plant stems and root systems have to be removed and examined (Johnson and Russell, 2019), by manually extracting and shaking vigorously with water on a white tray to dislodge attached larvae (Batzer, 1993). Larvae and pupae collected must be transported to the laboratory for species identification. In the laboratory they must be transferred carefully with a pipette to a container with water from the site. Identification can be either performed after rearing the larvae in a breeder, or directly identifying the fourth instar using taxonomic keys (Becker et al., 2020; ECDC, 2018).

I.4.1.3. Sampling adult mosquitoes

There are a wide variety of sampling methods to collect adult mosquitoes. Depending on whether host-seeking or gravid females are targeted, different techniques can be used. However, some unspecific trapping techniques are available. Truck traps are nets installed in the roof of a vehicle. The net is open pointing forward and has the shape of a funnel that leads into a container where the sample is collected. Using this method, mosquitoes can be captured while flying and is useful to determine mosquito flight periods (ECDC, 2018). On the other hand, an electrocution trap consists of an electrified wire grid so when a mosquito collides with it, it gets killed and is collected (Becker et al., 2020). Malaise trap is a tent-like structure made with a net that ends with a funnel and a collecting jar on the top of the tent and that usually contains a killing agent. Insects flying into the trap get captured when trying to escape. Limitations with these types of traps include catching the populations that fly around them, or in the case of truck traps the terrain and volume of air covered with the trap, collecting, and killing other insects that do not belong to the Culicidae family (Silver, 2008).

Host-seeking females can be caught using a bait, host or kairomones that release stimuli and attract them. Female mosquitoes react to compounds such carbon dioxide exhaled by the host and odour components such as lactic acid emanated from the host sweat. They can as well respond to body heat and water vapour (Becker et al., 2020). Human landing collection (HLC) is a long-established method of collecting host-seeking females. It consists of attracting the females by exposing the whole body or only part of the body (leg or arm) and then collecting them with a mouth aspirator. To assess the population, females landing on humans is counted in an interval of time. It is a time-consuming method and there is risk of becoming infected, so it is not recommended in transmission areas. Also, depending on the attraction and the skills of the collector results may differ in the abundance estimation (Becker et al., 2020; ECDC, 2018). Moreover, the time of the day, the season and the location are also key for the trapping of the target mosquito species. In fact, this method is widely used for the collection of anthropophilic mosquitoes as an indicator of risk of disease transmission (Service, 1977). For example, this technique has been used with the mosquito vector *Ae. aegypti* to monitor their abundance (Marquetti et al., 2000) and to study their diel periodicity (Corbet and Smith, 1974).

Animal-baited traps can also be used for important vector species as they also feed on animals. However, depending on the mosquito species their host preference must be considered, as for example *Cx. pipiens* is ornithophilic and anthropophilic (Martínez de la Puente et al., 2016) whereas *Cx. territans* generally feed on amphibians (Eubanks et al., 2008). Therefore, this type of trap can use a wide variety of animals, such as rodents, cattle, goats, pigs, horses, or birds. As described in the HLC method, mosquitoes can be collected using an aspirator. This method is commonly used in WN surveillance studies (Becker et al., 2020; ECDC, 2018).

Suction traps supplemented with different attractants such as light (incandescent or UV light) (Moore et al., 2001), carbon dioxide or including both are the ones generally used to collect mosquitoes (McNelly, 1989). Many light traps have been developed and tested to attract and catch different mosquito species. Light traps are more effective in sampling areas with little light pollution and at night (ECDC, 2018). However, they have a lot of by-catch of other insect species and are less effective during the diurnal and crepuscular activity of mosquitoes (ECDC, 2018). By baiting light traps with carbon dioxide their efficiency is increased as it is a highly effective attractant for host-seeking females, as it emulates the breath of a mammal (McNelly, 1989). Carbon dioxide can be added through a tank or with dry ice, but a great disadvantage is its accessibility. Moreover, traps only baited with carbon dioxide (CO_2) do not attract male mosquitoes, which are attracted by the light of the trap. On the other hand, carbon dioxide attracts other hematophagous insects like sandflies (Phlebotominae), biting-midges (Ceratopogonidae) or black flies (Simuliidae). Mosquitoes, attracted by either by light, CO_2, or both, are sucked downward due to the airflow created by the fan and are collected in a container. Traps are very portable and easily powered by batteries (Becker et al., 2020; ECDC, 2018).

Other novel suction traps are the Mosquito Magnet, BG-Sentinel and Biogents Mosquitaire traps. BG-Sentinels and Mosquitaire traps are especially designed to trap *Ae. aegypti* and *Ae. albopictus* as they attract them with special chemical lure (BG-lure). They can operate either continuously through power supply or with a 12-volt battery (for a limited time). In addition, carbon dioxide can be implemented thus increasing the effectiveness as it will attract a wider range of mosquito species (e.g., *Cx. pipiens* s.l.) (ECDC, 2018). Mosquito Magnet traps are also CO2-baited which is produced by a propane container. Moreover, other chemical attractants such as octenol (1-octen-3-ol) or Lurex (L-lactic acid) can as well be added. These traps can be place in remote locations as they run continuously for up to 21 days, when the propane and octenol must be replaced. The disadvantages of this trap are that it is quite large, heavy, and expensive (Becker et al., 2020; ECDC, 2018). This trap can be used for the monitoring of *Anopheles* mosquitoes in remote locations (Rubio-Palis et al., 2012) and has been successfully evaluated for the surveillance and catching of a wide range of mosquito species (Dennett et al., 2004).

Gravid females searching an oviposition site can be captured either with gravid or sticky traps. Gravid traps consist of a black container filled with water, an infusion of dead leaves or hay. These traps are used in arbovirus surveillance programmes as there is a higher probability to detect viral infections in blood-fed mosquitoes. They are not considered very effective to attract *Aedes* mosquitoes. Sticky traps also attract gravid females and after landing they end stuck (ECDC, 2018).

Finally, to catch resting females, or adult mosquitoes resting in vegetation and in or around buildings a sweep net or an aspirator can be directly employed. Sweep netting and aspiration collects mosquitoes present in the intermediate vicinity and in a much more limited timeframe than suction traps. It can be useful for the study of other behavioural or biological categories of adult mosquitoes, such as overwintering, non-seeking adults or male individuals (Holderman et al., 2018). One of the advantages of using a sweep net or an aspirator is that is it quick and easy to operate, however a disadvantage is that it is a time-consuming technique. Moreover, depending on the operator performing the sweep netting sampling, this may yield very variable results. Therefore, it is very important that the sampling must be done by the same operator, and it must be standardized. Standarization of the sweeping netting and aspiration can be done either by fixing a period or by fixing the length of a transect (Hoick and Meek, 1991). These two techniques are useful for the capture of males, and some species are not attracted by light traps (Y.-C. Chen et al., 2011), like for example *Cx. quinquefasciatus* which seems to be repelled by light (Kline et al., 2006). Moreover, backpack aspirators are very useful to catch blood-fed females resting, thus for the identification of the host they had fed on (Schönenberger et al., 2016).

I.4.2. Laboratory research techniques

I.4.2.1. Rearing mosquitoes

Sometimes to identify a mosquito species it is necessary to rear the immature stages into adults. A mosquito breeder is used, and it consists of two clear plastic containers connected with a screw lid and a funnel. On the bottom container, the water and the immature stages are placed, and when the adults hatch, they fly to the top container through the funnel. The larvae or pupae collected in the field must be kept in the water from their breeding site to facilitate its development. Water from the breeding sites contains food, however if there are a great number of immature stages then distilled water is used and small quantities of food must be added, such as dried yeast, powdered fish food or liver powder. To avoid anaerobic conditions due to an excess of food or scum accumulation, then the water must be changed. Depending on the species, temperature requirements may vary but they can be kept in a range between 20 and 25 ºC (Becker et al., 2020).

I.4.2.2. Morphological identification

Mosquito species identification is performed using morphological characteristics following dichotomous keys (**Figure 4**). Adult male mosquitoes are identified through their genitalia, whereas for females the whole specimen is needed. Species identification can also be carried out using the fourth instar larva. There are several taxonomic keys available like the ones from Becker et al. (2020), Wilkerson et al. (2021), Schaffner et al. (2001) and Gunay et al. (2020), the last two being interactive. This technique requires expertise and experience and is time-consuming. Moreover, it gets even more problematic when the specimen is damaged.

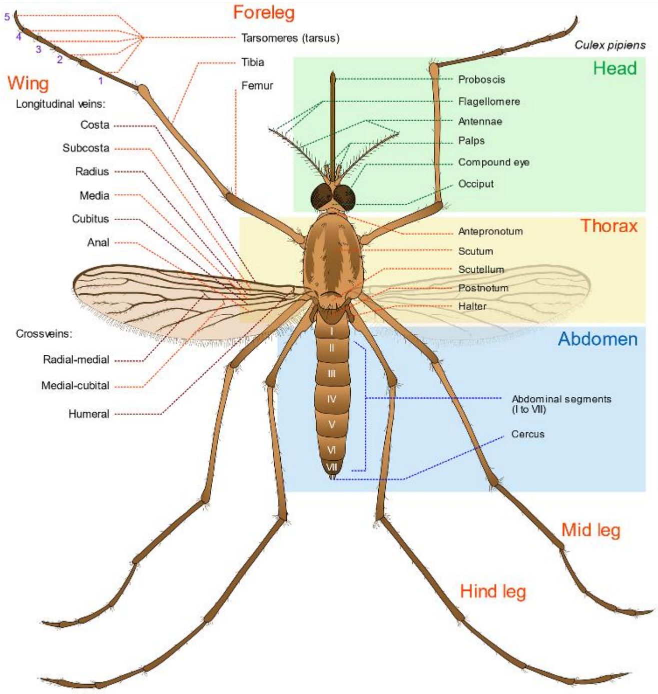

Figure 4. Morphology of an adult mosquito.

Diagram by Mariana Ruiz Villareal

Males are easily differentiated from females as most of them have plumose antennae and hairy maxillary palps as long as the proboscis. Only in the *Anopheles* genus, female's maxillary palps length is the same as the proboscis. The head, the thorax and the abdomen are covered with scales and setae, the distribution pattern, as well as their extension and colour are genus specific. The scutum, as well, can be covered with broad or narrow scales that covers all or only some areas, occasionally forming different patterns with colour scales. These patterns sometimes play an important role in the identification of the species, thus being important the management of the specimen in order not to lose these characteristics. Normally, the abdomen is covered dorsally and abdominally in scales in the Culicinae subfamily. However, in the Anophelinae subfamily, scales are absent. The morphology of the male genitalia is key for species identification (Becker et al., 2020; Service, 2012).

The dorsal edge of the scutellum can be either evenly convexed, as in the *Anopheles* genus, or three-lobed, as in the Culicinae subfamily. Narrow or broad scales may cover the whole scutum or only some of its areas, and the amount of scaling varies between different genera. The scales may be all the same colour on the entire scutal surface or differently coloured producing usually linear, longitudinal, and patch-like scale patterns which are often species-specific and consequently crucial for identification (Becker et al., 2020). The scales of the legs sometimes form patterns with different colours, usually in the form of a ring, which helps identifying the diverse species. Wings are also covered with scales and their colour and patterns differences are species-specific. In the posterior border of the wing, scales form a fringe, which is specific of the Culicidae family (Service, 2012).

For the identification of the larvae, the head of the two subfamilies (Culicinae and Anophelinae) differ in shape, as the head in culicine larvae are wider than longer, whereas anopheline larvae usually have longer than wider heads. On the dorsal surface of the eighth segment of the abdomen, the siphon is located, except in the Anophelinae subfamily, where the siphon is absent. Instead, they have respiratory fossae in the abdomen and therefore they lie parallel to the water surface. Other morphological characteristics for species identification are the number and types of setae present in all the body of the larva (Becker et al., 2020).

As for pupae morphology, they have fewer morphological attributes for species differentiation than larvae and adults. Therefore, for species identification, it is easier to rear the pupae until adult emergence (Becker et al., 2020).

In order to identify a mosquito species using only morphological characteristics, as previously said, it is important to have detailed morphological keys, trained entomological technicians and a tentative knowledge of the species present in a region. However, when the morphological characteristics of a specimen are rubbed off or damaged then it can be impossible to identify them. Moreover, some related species or complexes cannot be separated just by morphological features. Then molecular methods are needed to differentiate these mosquitoes (Beebe, 2018).

I.4.2.3. Molecular identification

A universal method for species identification is the DNA barcoding and it is used when the specimen is damaged, or confirmation of identity is needed. This technique is based on the use of a short DNA sequence that has much less variance within species than between species. Presently, the most used barcode region is a 5'-segment of the mitochondrial gene cytochrome oxidase I (COI) (Hebert et al., 2003) which is the standard marker chosen by the Barcode of Life Database (BOLD), a platform for collating and curating DNA barcoding information (Ratnasingham and Hebert, 2007). The method compares the DNA extracted from the mosquito specimen to the DNA sequences available in a reference library such as GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) or BOLD Systems (Hernández-Triana et al., 2019). Other DNA regions also used for DNA barcoding are ND4 and ITS2 (Ibáñez-Justicia et al., 2020). The advantages of using the mitochondrial COI gene are: i) it is universal, as it is present in all eukaryotic organisms, ii) it has a high number of copies in the cell, which is good for PCR amplification, iii) it allows a good discrimination rate between species, iv) it is of maternal inheritance, thus providing an evolutionary history and describing biodiversity. However, it does not always discriminate between sibling and complexes of species, as these do not show enough variability for their individual classification, and therefore other techniques must be employed (Beebe, 2018).

Depending on which sibling, complex or ecoform species needs to be identified there are different target genes and techniques to apply. Analysis of the polymorphisms of the DNA through PCR amplification and fragment lengths are used. For the differentiation of *Anopheles maculipennis* complex, a Restriction Fragment Length Polymorphism (RFLP) PCR flanking the Internal Transcribed Spacer 2 (ITS2) of the ribosomal DNA allows the identification of four sibling species of this complex: *An. atroparvus*, *An. labranchiae*, *An. maculipennis* s.s and *An. melanoon* (Vicente et al., 2011). For the identification of members of the *Cx. pipiens* complex (*Cx. pipiens*, *Cx. quinquefasciatus, Cx. pipiens pallens* and *Cx. australicus*), as well as hybrids of *Cx. pipiens*, *Cx.*

quinquefasciatus and other sibling species commonly mislabeled *Cx. pipiens* like, *Cx. torrentium* and *Culex pervigilans*, two multiplex PCRs have been developed, the first one using the acetylcholinesterase 2 gene (ace-2) and the second one with the CQ11 gene. With the ace-2 multiplex PCR, depending on the primers used and the geographic region of the specimens collected, six species (*Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. torrentium*, *Cx. pervigilans*, *Cx. australicus* and *Cx. pipiens pallens*) and their hybrids can be distinguished (Smith and Fonseca, 2004). Whereas the CQ11 multiplex PCR distinguishes between *Cx. pipiens* s.l. ecoforms: *Cx. pipiens* form *molestus*, *Cx. pipiens* form *pipiens* and their hybrid *Cx. pipiens pipiens/molestus* (Bahnck and Fonseca, 2006). As for the differentiation of the mosquito sibling species of *An. claviger* complex, a conventional PCR targeting the ITS2 region of the ribosomal DNA has been designed allowing the identification of the two species belonging to this complex: *An. claviger* s.s. and *An. petragnani* (Proft et al., 2003).

Besides, some real-time PCR have been developed for the identification of sibling species and hybrids. For example, for the identification of *An. maculipennis* complex (Lühken et al., 2016), *Cx. pipiens* complex and their ecoforms (Rudolf et al., 2013; Vogels et al., 2015), and even for the differentiation and identification of AIM (Van De Vossenberg et al., 2015).

I.4.2.4. Blood-meal analyses

Identification of the hosts upon which female mosquitoes feed is crucial to understand the dynamics and interactions of vectors, hosts, and pathogens. In fact, it is important for the prevention of epidemics and outbreaks to know host preferences and feeding patterns of the different mosquito species (Fikrig and Harrington, 2021).

There are different methods that have been developed to identify the host species of which mosquitoes feed on. For example, serological techniques, molecular amplification and sequencing of the host DNA and mass spectrometry. DNA barcoding has been frequently employed, there are different genes that are amplified to identify the host to species level (Gutiérrez-López et al., 2022).

Serological techniques have been widely used to identify blood-meals of mosquitoes. These techniques include precipitin tests and passive hemagglutination. These techniques are based on conjugating the blood recovered from the mosquitoes with immunoglobulin (IgG) to different potential hosts. The big disadvantage of this technique is finding available specific anti-sera to all potential hosts. Moreover, cross-reactions can result with closely related species (Gutiérrez-López et al., 2022).

Currently, molecular techniques are more used as they can identify the vertebrate hosts up to species level. Most studies are based in DNA barcoding, and there are different target genes such as the 12S and 16S of the Mitochondrial Ribosomal DNA, NADH Dehydrogenase Subunit I (NDI), the cytochrome b (cyt b) and the cytochrome c oxidase subunit I (Cox1 or COI). The last one is the most widely used (Gutiérrez-López et al., 2022).

Mosquitoes tend to feed on different hosts, thus mixed blood-meals are frequent, therefore identification of mixed blood-meals are complicated because produce ambiguous DNA sequences with double peaks in the sequencing electropherograms (Reeves et al., 2018).

II. Background, hypothesis, and objectives

Culicidae species, besides being a nuisance due to their biting behaviour, pose a threat to humans and animals as vector of many diseases. This together with the expansion of the distribution of some species and climate change, has created a new scenario in Europe in which emerging vector-borne diseases are being transmitted (Giunti et al., 2023a). However, not only exotic invasive mosquito species are responsible, as native mosquitoes are also vectors of pathogens (Kampen and Walther, 2018). Therefore, it is important to know which species, native and invasive, are present in an area to assess transmission risk of MBD. Thus, surveillance programmes are crucial to manage and control both vectors and diseases (Kampen et al., 2015).

In Europe up until the 1980s the most important disease transmitted by mosquitoes was malaria (Zeller et al., 2013). Malaria was endemic and was eradicated using insecticides such as DDT, and by elimination of marshes and wetlands and prophylactic drug administration (Boualam et al., 2021). The current situation in Europe is defined as "anophelism without malaria", as the vectors of this disease are still present but not the parasite (Piperaki and Daikos, 2016). Although, autochthonous outbreaks have been reported in Greece in recent years (Olaso et al., 2017). Until the detection and expansion in Italy of *Aedes albopictus* in the 90s, the main concern regarding mosquito-borne diseases in Europe was the imported human cases of arboviruses linked to travel and migration (Kampen and Walther, 2018). However, now that *Ae. albopictus* is established in many European countries, imported human arboviruses are very worrying, as autochthonous outbreaks can happen. In fact, there have been several MBD outbreaks in Europe and worldwide, such as chikungunya (https://www.ecdc.europa.eu/en/chikungunya-monthly), dengue (https://www.ecdc.europa.eu/en/publications-data/countriesterritories-reporting-dengue-cases-february-2023-and-january-2024), or West Nile (https://www.ecdc.europa.eu/en/news-events/epidemiological-update-west-nile-virus-transmission-season-europe-2023-0). In Spain the scenario is similar. Malaria was also endemic and eradicated in 1964 being considered its main vector *An. atroparvus* (Bueno Marí and Jiménez Peydró, 2008). Moreover, YF and dengue were also circulating in southern areas of Spain, with severe outbreaks reported in the 18th and 19th century, until the disappearance of the vector *Ae. aegypti* (Eritja et al., 2005). *Aedes albopictus* was detected for the first time in Spain in 2004 in Catalonia, after complaints of residents about insect bites in the area (Aranda et al., 2006; Giménez et al., 2007). Therefore, the Spanish Ministry of Health funded an entomological surveillance programme in 2007 with the objective to detect *Ae. albopictus* in possible points of entry, such as ports and airports, in the Spanish territory (Collantes et al., 2015). Since the first detection of this vector species, it has been reported in other Spanish regions like Valencian Community, Balearic Islands, Region of Murcia, Madrid, Aragón, Andalusia, Navarra and the Basque Country (Collantes et al., 2015; Ministerio de Sanidad, 2023). Besides, *Aedes japonicus* was detected in northern Spain, in the autonomous region of Asturias in 2018 through a citizen science report in the Mosquito Alert platform (Eritja et al., 2019). In 2020 it was detected as well in two other adjacent regions, Cantabria and Basque Country (Eritja et al., 2021). Moreover, *Ae. aegypti* was found in 2017 the Canary Islands, specifically in the island of Fuerteventura, through the entomological surveillance programme of the Ministry of Health, mentioned above, but this introduction was eliminated (Barceló et al., 2022). However, it was again detected in 2022 in the island of La Palma (Centro de Coordinación de Alertas y Emergencias Sanitarias, 2022), and in Tenerife (Centro de Coordinación de Alertas y Emergencias Sanitarias, 2023a). In Catalonia, local *Ae. albopictus* mosquitoes were found infected with dengue virus around a dengue viraemic case from an infected traveller in 2015 (Aranda et al., 2018). Autochthonous cases of MBD have been also reported in Spain, like the autochthonous malaria transmission in 2010 (Santa-Olalla Peralta et al., 2010), the autochthonous dengue cases detected firstly in 2018 in Murcia (4 cases) and Madrid (1 case) (Monge et al., 2020), and in 2019 (one in Madrid and one in Cataluña) (Centro Nacional de Epidemiología. Instituto de Salud Carlos III., 2022). In Ibiza two cases were reported in 2022 (Centro de Coordinación de Alertas y Emergencias Sanitarias, 2023b). Besides, WNV infection appeared in southern Spain in 2010 (García-Bocanegra et al., 2011), in 2016 (López-Ruiz et al., 2018) and in 2020, when an extensive outbreak occurred (Figuerola et al., 2022).

Globalization together with climate change affect the distribution of Culicidae species, introducing new species of mosquitoes to other regions of the world (Lühken et al., 2023). Furthermore, as the climate is getting warmer and drier in Europe invasive mosquitoes will encounter a more suitable environment to thrive (Cunze et al., 2016; Iwamura et al., 2020), even affecting the reproduction rate of the pathogens they transmit (Kilpatrick and Randolph, 2012; Mora et al., 2022). Landscape anthropization and urbanization also impact mosquito community composition (Ferraguti et al., 2016), by increasing or decreasing the species richness or the abundance of several mosquito species in urban environments (Gangoso et al., 2020; André B. B. Wilke et al., 2021)(Wilke et al., 2021). Therefore, knowing the distribution and abundance of native and exotic mosquitoes in different regions and environments is key to assess Public Health risks (ECDC, 2014).

Among native mosquitoes, *Cx. pipiens* s.l. is the most cosmopolitan species in the Northern Hemisphere, especially in urban areas (Haba and McBride, 2022). Even though this species can be vector of WNV (Soto et al., 2023), other *Culex* species that are present in Europe, including Spain, are vectors as well (Ciota, 2017). In fact, *Cx. modestus*, which has a wide distribution in

Europe, is involved in the transmission of WNV in the continent (Ciota, 2017). Moreover, *Cx. perexiguus* has been identified as the most important species in the transmission of WNV in southern Spain (Figuerola et al., 2022) due to its wide distribution and abundance in that area (Ferraguti et al., 2021). Other important native mosquito species in Europe due to their biting behaviour and disease transmission, are *An. maculipennis* s.l*., An. plumbeus*, *Ae. caspius*, *Ae. detritus* and *Ae. vexans*. Regarding *An. plumbeus*, it is a widely distributed species around Europe (Bueno-Marí and Jiménez-Peydró, 2011), and it has gained interest as it has adapted to new rearing sites in man-made containers which is causing larger population abundance and closer distribution in human areas, that together with its capacity to transmit malaria it could pose a risk to Public Health (Bertola et al., 2022). *Aedes caspius* and *Ae. detritus* are anthropophilic mosquitoes that cause nuisance in coastal and marshland areas in Europe (Veronesi et al., 2012). Moreover, these two species together with *Ae. vexans*, *Cx. pipiens* s.l. and *Cx. theileri* are potential vectors of Rift Valley fever virus (Drouin et al., 2022). In addition, *Cx. theileri* has a role in the transmission of *D. immitis* in the Canary Islands (Martínez de la Puente et al., 2012).

Some regions of Spain are understudied for Culicidae species, especially in the north of Spain. Due to the climatic factors the risk for the transmission of MBD has been suspected to be lower than in southern regions (Miró et al., 2013). However, prior to the start of this thesis, there were a few studies about the native culicids present in the Basque Country (González et al., 2016, 2015) that were carried out in specific locations. For instance, in the study of González et al. (2015) only four Culicidae species were identified in an urban park of the city of Vitoria-Gasteiz (*Cx. pipiens* s.l., *Cx. hortensis*, *Cs. longiareolata*, and *Cs. litorea*). The second study (González et al., 2016) was performed in a farm and a total of 602 culicids belonging to four different genera (*Culex*, *Culiseta*, *Anopheles* and *Aedes*) were trapped. Our **hypothesis** at the moment of planning the research to be carried out in this thesis was that in the Autonomous Region of the Basque Country, the number of Culicidae species is higher than the previous studies have suggested, as the geography of the Basque Country is varied, and in addition to urban areas, where a reduced number of species is estimated due to the anthropization effect, also includes natural areas with habitats that might harbour a great richness of mosquito species.

Regarding IMS, the entomological surveillance programme of the Basque Country detected *Ae. albopictus* for the first time in the year 2014. Since then, this species has shown a progressive increase, detecting its presence in the three Basque provinces five years later (Goiri et al., 2020). During this period, the presence of other AIM was not detected in the Basque Country but given the expansion of other invasive species in Europe (Medlock et al., 2015), the **hypothesis** proposed for this thesis was that besides *Ae. albopictus*, present in urban areas with heavy traffic, other invasive *Aedes* species could be present in other suitable types of environments according to the degree of urbanization.

Blood feeding preferences in mosquitoes have complex patterns as some species feed on a wide range of hosts. However, some species prefer to feed on a particular host or animal species, while others are generalists, or opportunistic, feeding on a wide range of available host species. Sometimes it is difficult to classify mosquito species in a single category as it can depend on host availability in the environment (Fikrig and Harrington, 2021). Some mosquitoes have a preference to feed on humans like *Ae. aegypti* and *Ae. albopictus* (Reinhold et al., 2018), whereas feeding preferences of the *Cx. pipiens* complex vary between ecoforms, for example *Cx. pipiens pipiens* has been described mainly as ornithophilic, while ecoform *molestus* feeds on humans (Osório et al., 2014). The selection of the host to feed on is crucial for the development of the life cycle of the pathogens. Therefore, without knowing the species of Culicidae that could be found in natural and urban areas, the starting **hypothesis** was that the Culicidae species of the Basque Country feed on the most abundant fauna of each type of environment.

Having considered the importance of the issue for Public Health and Animal Health, and the hypotheses proposed, the objectives of the thesis are as follows:

1. To identify the Culicidae species present in the Basque Country in different environments (natural, rural, periurban and urban), and the factors affecting their presence, abundance, and richness.

2. To study the distribution of *Aedes* spp. invasive species in the Basque Country according to different degrees of anthropization, and evaluate the factors involved in their presence.

3. To know the feedings patterns of mosquito species along an urban-to-wild gradient.

III. Materials and Methods

III.1. Study Area: The Basque Country

The Basque Country (42°78′ N, 02°44′ W), is a small region located in northern Spain and has an extension of *ca.* 7200 km^2. It is divided into three administrative provinces: Gipuzkoa (in the northeast), Bizkaia (in the northwest), and Araba (in the south). The climate is warm and temperate. The average annual temperature and rainfall are 13.4 °C and 1,610 mm in Gipuzkoa, 13.8 °C and 1,278 mm in Bizkaia, and 11.5 °C and 878 mm in Araba, respectively (De León et al., 1989).

The population of the Basque Country is *ca*. 2,188,017 inhabitants, and industry and tourism are some of the driving forces of the Basque economy (EUSTAT, 2020). Due to its geographical location, there is a network of highly trafficked motorways structured around the main routes that connect the region with France, central Spain, and the Mediterranean coast.

III.2. Sampling Design

Depending on the objectives and type of area sampled different sites were selected and there will be described below.

III.2.1. Study I

The first study was carried out in two natural aquatic environments in the Basque Country. The wetland of Salburua (Alava province; 42°51′N, 02°39′O) and the marsh of Urdaibai (Biscay province; 43°22′N, 02°40′E) are two protected water environments that provide important wintering and migratory resting places for a wide range of birds' species. Salburua wetland extends 217.46 ha and comprises two main shallow lakes surrounded by meadows and a small oak grove. This wetland is an important habitat for many wildlife species, being the birds the most frequent host community. Walkers accompanied by dogs are also seen. Climate oscillates during the year, with a dry and hot summer and a wet and cold winter. In consequence, the lagoons completely full during the winter dry gradually and completely in summer. The marsh of Urdaibai is located on the Bay of Biscay. It covers a vast area of around 220 km^2 consisting of marshlands, meadows, green oak forests, and leafy woods. This high preserved environment host hundreds of vertebrate species, mainly birds, mammals, and humans. It has a temperate and humid climate, with frequent rainfall even during the summer months. The marsh remains flooded throughout the year, with oscillations during the summer and depending on the location, the marsh water is either fresh or salty.

Two sampling sites were selected in each natural area in 2018 (July-October) and in three sites in 2019 (May-October). In 2018 and 2019, mosquito captures were carried out fortnightly using CDC traps with CO_2 and a sweep net. In 2019, at a monthly basis, mosquito breeding sites were examined for larvae collection in the vicinity of the CDC traps, and host-seeking females approaching the operator were also collected to identify human landing Culicidae species. In addition, mosquitoes’ feeding habits were studied by analysing host blood in fed and gravid females.

III.2.2. Study II

The study was conducted on three municipal Animal Protection Centres (APC) located in the vicinity of the three provincial capitals (APC1, Vitoria-Gasteiz – Araba; APC2, Bilbao – Bizkaia; and APC3, Usurbil – Gipuzkoa) of the Basque Country. The mountainous terrain defines distinctive climate areas. APC1 is in the central-southern area of the Basque Country, influenced by a continental climate with cold winters and warm and dry summers while APC2 and APC3 are in the green northern region, influenced by an oceanic climate with wet weather and mild temperatures all year round. The three APCs showed distinctive environmental characteristics, pet census and geographical locations. Whereas APC1 (42.832882, −2.711116) and APC2 (43.273048, −2.908081) were in periurban areas, APC3 (43.294444, −2.065416) was in a rural area. All three APCs were erected on concrete floor with limits fenced by open-metal panels allowing the free entrance of external insects. Other facilities within the premises included green yards, vegetation patches and veterinary practice areas. The surroundings varied among studied sites, e.g., APC1 was surrounded mainly by green areas and field crops, whereas APC2 and APC3 were bordered by woodlands.

Four CDC light traps (2 incandescent and 2 UV) were deployed in each APC to catch mosquitoes. Trapping took place between 1 July and 31 October 2018. A total of eight samplings (one sampling every 15 days) were undertaken at each APC.

III.2.3. Study III

A cemetery and an urban green area were selected in the three main cities of the Basque Country. In Vitoria-Gasteiz (inland city) the cemetery of Santa Isabel and the municipal plant nursery. The cemetery of Santa Isabel is a small cemetery located in the heart of the city, its cemented areas are mixed with trees and green patches, making it a naturalized area. The municipal plant nursery is in the outskirts of the city, with green patches, trees shrubs and a couple of greenhouses. In Donostia-San Sebastián (coastal city), the municipal cemetery (Polloe) was sampled together with Kristinaenea Park. The cemetery of Donostia is significantly cemented with few green areas, and the tombs and monuments are quite compacted together.

Kristinaenea Park is situated in the centre of the city, it is a very naturalized green area, with a lot of vegetation, variety of animals species, and swamps. It is a frequently visited by school, families and citizens to enjoy the nature and different recreational and physical activities. On the other hand, in Bilbao (estuarine city), the municipal cemetery of the city and Doña Casilda Park were sampled. The municipal cemetery of Bilbao is located in a rural area 10 km from the city. It is quite large, not very compacted and the tombs and monuments are surrounded by grass and shrubs, but there are not many trees. Doña Casilda Park is in the city centre, it has a central pond with different aquatic bird species, green patches, and different species of trees. There are abundant cemented areas, and it is frequently visited by citizens and families to enjoy different recreational, physical and leisure activities.

The samplings were carried out fortnightly during two periods: from May to October 2019 and from June to November 2020 (due to COVIC-19 pandemia). Two CDC traps were deployed in each sampling site. In addition, in 2019 mosquito breeding sites were searched and sampled once per month in a radius of 200 m around CDC traps. In addition, mosquitoes' feeding habits were studied by analysing host blood in fed and gravid females collected in 2019.

III.2.4. Study IV

For the study of the distribution of IAM' eggs, the sampling strategy in 2021 encompassed the placement of ovitraps (ECDC, 2012) in 45 municipalities with more than 10,000 inhabitants. Two sampling zones were selected in each municipality, except in the three main cities (Bilbao, Donostia/San Sebastian and Vitoria-Gasteiz), where the number of selected zones increased to 8, 17, and 4, respectively. A total of 113 sampling areas were selected. Sampling started on June 1st and finished on November 18th. Thus, each municipality and area were sampled from 11 to 12 times over a period of 23 weeks (June-November). Five ovitraps were placed in each sampling area, always in shady places and hidden in the vegetation.

Information on each sampling area was compiled, including geographic coordinates and type of environment (parking, green park, petrol station, city center, industrial zone). Sampling sites were also categorized according to the urbanization degree: urban, suburban, and peri-urban areas according to Loibl et al (2011).

III.3. Methodology

III.3.1. Collection of mosquitoes with CDC traps

CDC miniature traps (model 1212, John Hock, USA) equipped with incandescent light (Studies 1, 2 and 3) and UV light (Study 2) and baited with ca. 1.5 Kg of dry ice (CO_2) were used.

The traps were deployed at each site fortnightly for 24 h (set early in the morning and recovered the next day) suspended on tree branches at 1-1.5 m and protected against sunlight and wind exposure (**Figure 5**).

Figure 5. CDC traps baited with CO_2

III.3.2. Collection of immature stages

Potential larval rearing sites (stagnant water ditches, ponded streams, puddles, artificial containers, edge of water bodies, and aquatic shallow areas, among others) were selected to be sampled around 200 m radius for each CDC-trap position (Studies 1 and 3). The selected larval habitats were systematically sampled once per month in the morning by dipping (600 ml-cup dipper) (**Figure 6**) during 15-20 min providing enough time for the active search, collection of immature stages and suitable estimation of the mosquito community (Bueno-Marí, 2010; González et al., 2021). In the field, the collected material was transferred into 500 mL-plastic flasks on its own water and transported to the laboratory as soon as possible.

Figure 6. Method of dipping in larval habitats

Then, immature stages were transferred to mosquito breeders (Bioquip, USA) (**Figure 7**) and reared over a maximum of 3 weeks to obtain adult mosquitoes under room temperature (20-22º C) and low light intensity. Upper portions of the mosquito breeders with emerged adults were stored at -30ª C for further morphological identification.

Figure 7. Mosquito breeders

III.3.3. Human landing collection

Host-seeking female mosquitoes landing on the operator were collected by aspiration (pooter style aspirator, Bioquip, USA). The operator examined the presence of mosquitoes on the exposed and non-exposed human parts. Human landing collections (HLC) (Figure 8) were conducted during 15 min during the morning period (10.00-12.00) near to the CDC-traps during the examination of larval breeding sites. The catches were stored dry at -30º C until further morphological identification as explained below.

Figure 8. Mosquito landing on the operator

III.3.4. Collection of resting mosquitoes by sweep-net

Culicidae resting on vegetation were collected with a polyester long-handled net (38 cm diameter, 0.8 mm mesh size, Bioquip, USA) between 9.30 and 11.00 a.m. The net was swept for approximately 4 min over the surface of herbaceous and shrubby vegetation around a 25 m radio area from each CDC-trap position. Collected mosquitoes were transported to the laboratory and immediately stored at -30 ºC, to kill them before their morphological identification.

III.3.5. Ovitraps for *Aedes* eggs sampling

Ovitraps consisted of a dark container (250 mL) filled with non-chlorinated water and a wooden stick (15 cm long and 2 cm wide) submerged inside as an oviposition substrate. Ovitraps were placed in each sampling site in wind-protected shaded areas (Figure 9); in many cases, surrounded by vegetation; and near walls or fences. Ovitraps within each sampling site were separated by at least 15–20 m.

Figure 9. Ovitrap with an oviposition substrate inside (masonite)

Ovitraps were examined every 15 days, and the wooden sticks were replaced and transported to the laboratory for examination. The water content of the ovitraps was also visually examined for mosquito larvae, and the water was then replaced using non-chlorinated water. In the laboratory, each labelled wooden stick was studied under a stereoscopic microscope and the eggs with morphology compatible with *Aedes* mosquitoes were counted (Figure 10). A selection of sticks from positive sites were immersed in Petri dishes with non-chlorinated water for hatching of the fourth stage larvae (L4), and then transferred to mosquito breeders (Bioquip, USA) to molt to adult mosquitoes (**Figure 7**).

Figure 10. Invasive *Aedes* spp. eggs observed under a stereoscopic microscope

III.3.6. Morphological identification of mosquitoes

In the laboratory, mosquitoes were separated by sex, and females were classified by their physiological status (blood-fed, gravid, and unfed). Species identification was based on morphological features of females and male genitalia (mounted in slides with Hoyer's medium) using taxonomic keys (Becker et al., 2020; Gunay et al., 2020; Schaffner et al., 2001). For further identification of blood-meal host species, abdomens of blood-engorged and gravid females were removed (Studies 1 and 3) and individually stored in 2 ml screw-top vials at -20ºC after being classified according to the scale of Sella (II-VII), by observation of the degree of digestion of the blood and developing eggs (gravid stage = Sella stage VII) (Detinova, 1962).

III.3.7. Molecular identification of mosquitoes

Damaged or morphologically indistinguishable mosquito specimens were identified by molecular methods. Firstly, genomic DNA extraction was carried out with NZY Tissue gDNA isolation kit (NZYTech, Lisboa, Portugal). For the sample preparation, the abdomen of the mosquito was used (if not available, the torax or the legs). The abdomens were cut with a scalpel and placed in 2 mL screw tubes with 200 µl of TE buffer and 2.3 mm diameter steel beads. The tissue was then homogenized using the TissueLyser (Qiagen, Hilden, Germany) at a frequency of 30 s^{-1} for 20 minutes. Afterwards, 180 µl of NT1 buffer and 20 µl of Proteinase K was added, and the sample was digested at 56 ºC for 2 hours. The next steps of the extraction followed the manufacturer's instructions, but DNA was eluted in 100 µl of elution buffer instead of 200 µl.

A PCR targeting the Cytochrome c oxidase I subunit (COI) gene, using the primers C1-J-1718 (5‘ – GGAGGATTTGGAAATTGATTAGTGCC – 3’) and C1-N-2191 (5’ – CCCGGTAAAATTAAAATATAAACTTC – 3’) described in Delgado-Serra et al. (2021) and Simon et al., (1994). The 25 µl PCR reaction consisted of 12 µM of each primer, 0.2 mM of dNTP, 3 mM of Mg^{2+}, 0.4 mg/ml of BSA, 1x reaction buffer, 1 unit of DNA polymerase (Life Technologies, Carlsbad, CA, USA) and 4 µl of DNA. The PCR conditions are detailed in Table 1.

COI PCR Conditions		
Temperature	**Time**	**Cycles**
94ºC	1 min	
94ºC 45ºC 72ºC	1 min 1 min 30 s 1 min 30 s	x 5 cycles
94ºC 57ºC 72ºC	1 min 1 min 30 s 1 min	x 35 cycles
72ºC	5 min	

Table 1. Conditions for the PCR targeting COI region.

Afterwards, PCR amplicons were purified using ExoSAP-IT (Applied Biosystems, Thermo Fisher Scientific, Vilnius, Lithuania) and submitted for Sanger sequencing (Eurofins Genomics, Germany). The sequences obtained were analysed using BioEdit software (v.7.2.5) (Hall, 1999), and compared with the GenBank database by nucleotide sequence homology searches at the network server of the National Center for Biotechnology Information (NCBI) using BLAST or at the Barcode of Life Database (BOLD) (http://www.boldsystems.org/index.php).

DNA of specimens identified as *An. maculipennis* s.l. were identified using a PCR-RFLP assay targeting polymorphisms in the Internal Transcribed Spacer 2 (ITS-2) of the ribosomal DNA. ITS-2 primers used were 5.8S (5’–TGTGAACTGCAGGACACATG-3’) and 28S (5’–ATGCTTAAATTTAGGGGGTA–3’) (Collins and Paskewitz, 1996; Vicente et al., 2011). The 25 µl PCR reaction mix consisted of 5 µM of each primer, 0.2 mM of dNTP, 2.5 mM of Mg^{2+}, 0.4 mg/ml of BSA, 1x reaction buffer, 1 unit of DNA polymerase (Life Technologies, Carlsbad, CA, USA) and 4 µl of DNA. The PCR conditions are detailed in Table 2.

ITS-2 PCR Conditions		
Temperature	**Time**	**Cycles**
94ºC	5 min	
94ºC	30 s	x 34 cycles
53ºC	30 s	
72ºC	30 s	
72ºC	7 min	

Table 2. ITS-2 PCR conditions

Afterwards, PCR amplicons were digested first with *HhaI* and secondly with *HpaII* restriction enzymes (Invitrogen/Thermo Scientific, Vilnius, Lithuania) following manufacturer's instructions. The bands that can be obtained are 300 bp / 389 bp/ or two bands of 108 and 135 bp.

- *An.labranchiae* /*An.maculipennis* s.s. → 300 bp
- *An. atroparvus* → 389 bp
- *An. melanoon* → 108 and 135 bp

If bands at 300 bp *An.labranchiae* /*An.maculipennis* s.s. appear on the agarose gel, a second digestion with the enzyme *HpaII* must be performed to differentiate the two species.

Second digestion with *HpaII* enzyme: From the product of the first digestion of the amplicon, 10 µl are taken and 1 µl of the restriction enzyme *HpaII* is added.

Visualisation of PCR products: Digested amplicons were separated by 2% agarose gel electrophoresis using GelPremium (NZYTech, Lisboa, Portugal) as staining solution, and with a 100 bp DNA ladder (NZYTech, Lisboa, Portugal) as a molecular weight marker. Five µl of the product and a 100 bp marker are used.

After the first digestion, bands can be obtained:

- *An.labranchiae/An.maculipennis* s.s. → 300 bp
- *An. atroparvus* → 389 bp
- *An. melanoon* → 108 and 135 bp

After the second digestion the bands obtained would be:

- *An.labranchiae* → 279 bp
- *An.maculipennis* s.s. → 201 bp.

The specimens identified as *Cx. pipiens* s.l. were analysed by a PCR targeting the flanking region of the CQ11 microsatellite (Bahnck and Fonseca, 2006) to identify *Cx. pipiens* biotypes (*Cx. pipiens* form *pipiens*, *Cx. pipiens* form *molestus* and the hybrids). The primers used in the multiplex PCR were:

- CQ11F (5’ – GATCCTAGCAAGCGAGAAC – 3’)
- pipCQ11R (5’ – CATGTTGAGCTTCGGTGAA – 3’)
- molCQ11R (5’ – CCCTCCAGTAAGGTATCAAC – 3’)

The 25 µl PCR reaction mix consisted of 0.15 µM of the CQ11 primer, 0.10 µl of pipCQ11R and molCQ11R, 0.2 mM of dNTP, 2.5 mM of Mg^{2+}, 0.4 mg/ml of BSA, 1x reaction buffer, 1 unit of DNA polymerase (Life Technologies, Carlsbad, CA, USA) and 4 µl of DNA. The PCR conditions are detailed in Table 3.

CQ11 PCR Conditions		
Temperature	**Time**	**Cycles**
94ºC	5 min	
94ºC	30 s	x 40 cycles
54ºC	30 s	
72ºC	40 s	
72ºC	5 min	

Table 3. CQ11 PCR Conditions

Amplicons were then separated by 1.5% agarose gel electrophoresis using GelPremium (NZYTech, Lisboa, Portugal) as staining solution, loading 5 µl of the amplified product and with a 100 bp DNA ladder (NZYTech, Lisbon, Portugal) as a molecular weight marker.

The CQ11 primers amplify bands of 266 and 284 bp.

Cx. pipiens pipiens → 266 bp
Cx. pipiens molestus → 284 bp
Hybrid *pipiens/molestus* → 266 and 284 bp

III.3.8. Blood meal analysis

We established a collaboration with the Centre for Biodiversity Genomics, University of Guelph (Guelph, ON, Canada) to investigate vertebrate host species of blood-fed and gravid mosquito females. Briefly, abdomen was removed for DNA purification (Ivanova et al., 2006) and mosquito species identification was done by amplification of the entire barcode region of COI gene (Hernández-Triana et al., 2014), and amplicons were used for Sanger sequencing. Host feeding patterns were identified using a metabarcoding-like approach with next-generation deep sequencing technology (NGS) (Estrada-Franco et al., 2020; González et al., 2020). DNA was extracted using a modified glass fibre technique (Ivanova et al., 2006). The resulting DNA was used to ascertain the identities of the mosquito species as well as of the vertebrate hosts upon which they had fed. Mosquito species were identified using standard DNA barcoding techniques, employing universal insect primers (C_LepFolF+C_LepFolR) (Hernández-Triana et al., 2014) followed by Sanger sequencing. Traces were edited in CodonCode Aligner v9.0.1 and uploaded to the Barcode of Life Data System (BOLD). For vertebrate host identification, primers were designed to anneal to vertebrate but not insect DNA (C_BloodmealF1_t1 + Mod.Mamm.R_t1) (Estrada-Franco et al., 2020) followed by next-generation sequencing on an Ion Torrent S5 Sequencer (Termo Fisher Scientifc, Waltham, MA). The resulting sequence reads were processed by first removing reads with a quality score (QV) < 20. Following primer/adapter trimming, reads in the expected size range of 125–250 bp were clustered into operational taxonomic units (OTUs) with a minimum identity of 98%. OTUs represented by at least 10 reads were compared to a reference library consisting of all vertebrate COI barcode records on BOLD. Matches between an OTU sequence and a reference sequence were considered reliable only if at least 100 bp of the query sequence matched a reference with at least 95% homology. For each mosquito, all taxonomic matches were consolidated into a “unique taxonomic hit” table, with each hit supported by a total read count. Any taxonomic hits that occurred in negative control samples were proportionally subtracted from all other read counts, after which hits were only accepted as genuine if supported by at least 100 reads.

III.3.9. Data analysis

The free software R (version 3.6.1 and version 4.2.0) (R Core Team, 2022) was used to carry out the statistical analyses detailed in the different studies.

IV. Results

Study 1: Mosquitoes in natural environments in the Basque Country

IV.1.1. Background

Mosquitoes are blood-sucking Diptera of public health relevance because of their capacity to transmit pathogens to humans, wildlife, and livestock. The emergence or re-emergence of several diseases in Europe, including malaria, dengue, chikungunya, Zika, West Nile, and Usutu (Brugueras et al., 2020; Calzolari, 2016; García San Miguel Rodríguez-Alarcón et al., 2021; Vilibic-Cavlek et al., 2019), highlights the need to study mosquito species as potential vectors of the aforementioned pathogens in different scenarios, including natural habitats.

Wetlands are valuable environments that have gained interest because of the growing concern about mitigating climate change and preserving the biodiversity of fauna and flora. These strategies aim to expand these aquatic habitats to reduce the impact of rising sea levels and extreme flooding events, as well as restore drained wetlands worldwide (Dale and Knight, 2008; Medlock and Vaux, 2015a). However, these biotopes provide a wide variety of highly suitable larval sites for the development of immature stages of mosquitoes, which can have substantial health impacts on humans (Hawkes et al., 2020). In Europe, flooded ecosystems, such as freshwater habitats can favour the massive increase in endemic mosquito species, such as *Ae. caspius, Ae. vexans*, and *Ae. sticticus*, after natural or unnatural flooding events, which cause severe human nuisance (Dale and Knight, 2008; Medlock and Vaux, 2015a). Moreover, these environments are used by native and migratory birds as resting places in their migratory routes and therefore constitute potential areas of local or allochthonous zoonotic pathogen transmission (Dale and Knight, 2008; Jourdain et al., 2007; Roiz et al., 2015). The study of mosquito diversity and abundance is particularly interesting in freshwater settings (Roiz et al., 2015, 2012a), as many wetland habitats are visited by several bird species susceptible to carrying or transmitting the WNV (Jourdain et al., 2007) such as waterfowl, storks, raptors or passerines, among others. Studying the host-feeding habits of mosquitoes is therefore a critical step in understanding host-vector-parasite pathways, the role of the fauna as possible reservoirs of pathogens, and their transmission patterns (Rizzoli et al., 2015). Mosquitoes can have either a general preference or feed on a particular group of animals (Brugman et al., 2017); thus, the studies aiming to identify the host-feeding habits of potential vector species have a significant impact in evaluating mosquito vectorial capacity and understanding the transmission cycles.

In Europe, wetland ecosystems have been studied in relation to blood-sucking Diptera, their potential risk of spreading mosquito borne pathogens, and the impact they can have on these natural landscapes. Considering that several mosquito species can successfully transmit certain pathogens, monitoring of species present in these areas is of great importance for health surveillance and the implementation of appropriate management, if necessary, of these aquatic environments; so that potential disease risk can be balanced against the many environmental and societal benefits of wetlands (Hawkes et al., 2020). Therefore, in this study, we aimed to assess the community composition of mosquitoes in two aquatic ecosystems in northern Spain using different approaches for the collection of both adults and immature stages. Classical morphology, together with COI gene-based DNA barcoding tools, were used to identify mosquito species. Factors affecting abundance, as well as their host-blood meal sources, were investigated.

IV.1.2. Materials and methods

IV.1.2.1. Study area

The study was conducted in two aquatic environments in the Basque Country (**Figure 11**). The wetlands of Salburua (Araba; 42°51′N, 02°39′W; 512 m above sea level) and the marsh of Urdaibai (Bizkaia; 43°22′N, 02°40′E; 2 m above sea level) are two protected areas that provide crucial wintering and migratory resting places for a wide range of bird species. Both environments are frequently visited by students in schools, walkers, and ornithologists.

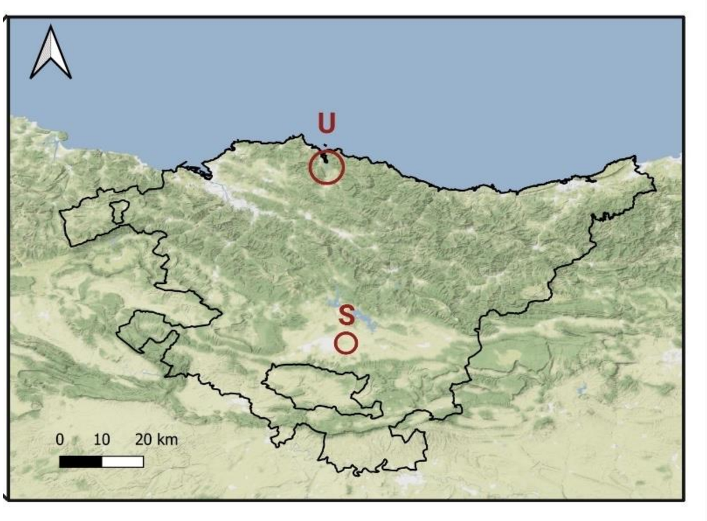

Figure 11. Location of the sampling sites. Map of the Basque Country indicating the two aquatic sites: Urdaibai (U) and Salburua (S).

Salburua is a natural wetland formed by the emergence of subterranean water from a Quaternary aquifer. It extends 2.2 km^2 and includes two main shallow water reserves of freshwater surrounded by meadows and a small oak grove. The wetland is partially bordered by a residential district of approximately 7,000 residents. Wetlands are predominant habitats for numerous wildlife species. The most frequent potential host communities are birds

(Anseriformes, Gruiformes, Passeriformes, and Ciconiiformes), mammals (Carnivora and Artiodactyla), and walkers accompanied by dogs. The climate oscillates during the year, with a dry and hot summer and a wet and cold winter; consequently, the lagoons are full during the winter and dry gradually in the summer (Frías-Saéz, 2022). During the course of the year, the temperature generally varies from 1 °C to 26 °C and rarely drops below -4 °C or rises above 34 °C.

The marsh in Urdaibai is located in the Bay of Biscay. It covers a vast area of approximately 220 km^2 and consists of marshlands, meadows, green oak forests, and leafy wood. Twelve municipalities are integrated in this territory, with approximately 45,000 inhabitants. This highly preserved environment hosts hundreds of vertebrate species, including birds (Anseriformes, Gruiformes, Passeriformes, and Podicipediformes), mammals (Carnivora, Artiodactyla, and Perissodactyla), and humans (ornithologists and walkers). It has a mild, temperate, and humid climate with frequent rainfall, even during the summer. During the year, the temperature generally varies from 6 °C to 24 °C and rarely falls below 1 °C or rises above 28 °C. The marsh remains flooded throughout the year, with periodic oscillations during the summer. Depending on the location, the marsh water is either fresh, salty, or mixed (Hernández and Caño, 1999).

IV.1.2.2. Sampling approach

Two sampling sites were selected in each natural area in 2018 and three in 2019. The collection sites consisted of woodlands, pastures, and vegetated ecosystems with permanent and temporary water bodies (lagoons, lakes, pools, ponded streams, slow flowing streams, and puddles). In 2018 (July–October, 8 samplings in each site) and 2019 (May–October, 13 samplings in each site), mosquitoes were captured using CO_2-baited CDC traps and a sweep net (SN). In addition, host-seeking female mosquitoes approaching the operator were collected to identify human landing species. In 2019 (May–October), mosquito larval sites were examined to collect immature stages in the vicinity of the CDC traps.

IV.1.2.2.1. Carbon dioxide (CO_2)-baited CDC-traps and sweep net (SN)

CDC miniature traps (model 1212, John Hock, Gainesville, FL, USA) equipped with incandescent light and baited with approximately 1.5 kg of dry ice (CO_2) were used. The traps were deployed at each site fortnightly for 24 h (one CDC trap per sampling site, set early in the morning and recovered the next day), suspended on tree branches at 1–1.5 m, and protected against sunlight and wind exposure. After placing CDC traps, Culicidae resting on the vegetation were collected with a polyester long-handled net (38 cm diameter, 0.8 mm mesh size, Bioquip, Compton, CA, USA) between 09.30 and 11.00 a.m. The net was swept for approximately 4 min over the surface of herbaceous and shrubby vegetation around a 25 m radius area from each CDC-trap position. The collected mosquitoes were transported to the laboratory and immediately stored at −20 ºC, to kill them before morphological identification.

IV.1.2.2.2. Human-landing catches (HLC)

Host-seeking female mosquitoes landing on the operator were collected by aspiration (pooter-style aspirator, Bioquip, Compton, NC, USA). An operator in the upright position collected mosquitoes from both the exposed and non-exposed human parts. HLC was conducted for 15 min during the morning period (10.00 a.m.–12.00 p.m.) near the CDC traps at the same dates as the examination of larval breeding sites. The catches were stored dry at −30 ºC until further morphological identification.

IV.1.2.2.3. Collection of immature stages

Potential larval developmental sites, including stagnant water ditches, ponded streams, puddles, artificial containers, edges of water bodies, and aquatic shallow areas, were sampled around a 200 m radius for each CDC trap position. The selected larval habitats were systematically sampled monthly by dipping (600 mL cup dipper) multiple times for 15–20 min in the morning which is considered an acceptable effort for the active search, collection of immature stages, and suitable estimation of the mosquito community (Bueno-Marí, 2010; González et al., 2021). Additionally, in those environments with aquatic vegetation (i.e. *Typha* sp.), roots were manually extracted and shaken vigorously with water on a white tray to dislodge attached larvae of the genus *Coquillettidia* (Batzer, 1993). A maximum of five root samples were extracted per date/habitat. In the field, the collected material was transferred into 500 mL plastic flasks with its own water (with aquatic vegetation if needed) and transported to the laboratory as soon as possible. Subsequently, the immature stages were transferred to mosquito breeders (Bioquip, Compton, CA, USA) and reared for a maximum of three weeks (fish food was provided in low amounts) at room temperature (20–22º C) and low light incidence to obtain adult mosquitoes. The upper portions of the mosquito breeders with emerged adults were stored at −20 ºC for further morphological identification.

IV.1.2.3. Morphological identification of mosquitoes

In the laboratory, mosquitoes were separated by sex and females were classified according to their physiological status (blood-fed, gravid, and unfed). Species identification was based on the morphological features of the female and male genitalia using taxonomic keys (Becker et al., 2020; Gunay et al., 2020; Schaffner et al., 2001). For further identification of blood-meal host species, abdomens of blood-engorged and gravid females were removed and individually stored in 2 mL screw-top vials at −20 °C after being classified according to the scale of Sella (II–VI) by observation of the degree of digestion of the blood and gravid stage (containing eggs) (Detinova, 1962).

IV.1.2.4. Molecular análisis

IV.1.2.4.1. Molecular identification of mosquitoes

Damaged or morphologically indistinguishable mosquito specimens (n = 104; 56.7% in Urdaibai and 43.3% in Salburua) were identified via molecular methods using the primers described by Delgado-Serra et al. (2021) and a PCR targeting the COI gene (Hernández-Triana et al., 2017). After DNA extraction from mosquito thorax using a commercial kit (QIAamp DNA Mini kit, Qiagen, Hilden, Germany), PCR amplicons were purified using ExoSAP-IT (Applied Biosystems, Thermo Fisher Scientific, Vilnius, Lithuania) and subjected to Sanger sequencing (Eurofins Genomics, Germany). The sequences obtained were analysed using BioEdit software (v.7.2.5) (Hall, 1999) and compared with the GenBank database by nucleotide sequence homology searches at the network server of the National Center for Biotechnology Information using the BLAST tool. Nucleotide sequence type (ntST) evaluation was carried out using DnaSP 6, and a phylogenetic tree was constructed by Neighbor-Joining (based on Kimura 2-parameters genetic distance model) with MEGA 6.0, resampled 1000 times to generate bootstrap values (Tamura et al., 2013).

DNA of those specimens identified as *An. maculipennis* s.l. upon sequencing (n = 6) were submitted to a PCR-RFLP assay targeting polymorphisms of the ITS-2 of the ribosomal DNA (Vicente et al., 2011) to identify individual members of the *An. maculipennis* complex. Specimens identified as *Cx. pipiens* s.l. upon sequencing (n = 22) were analysed using a PCR targeting the flanking region of the CQ11 microsatellite (Bahnck and Fonseca, 2006) to identify the *Cx. pipiens* female form (*Cx. pipiens pipiens, Cx. pipiens molestus*, and hybrids).

IV.1.2.4.2. Host blood-meal analysis

Vertebrate host species of blood-fed (n = 39) and gravid (n = 14) females were investigated at the Centre for Biodiversity Genomics, University of Guelph (Guelph, ON, Canada). The abdomen was removed for DNA purification (Ivanova et al., 2006) and mosquito species identification was performed by amplification of the COI gene (Hernández-Triana et al., 2014). The amplicons were submitted for Sanger sequencing as described in Estrada-Franco et al.

(2020) and González et al. (2020). Host feeding patterns were identified using a metabarcoding-like approach with NGS technology as previously described (Estrada-Franco et al., 2020; González et al., 2020). The raw sequence reads were filtered and cleaned. Reads passing these filters were clustered into OTUs with 97% identity and a minimum of 10 reads per OTU. Each OTU sequence was compared using the BLAST tool and BOLD Systems, which are composed of global vertebrate COI sequences. Identification was considered valid only when the query sequence matched the reference sequence with at least 95% nucleotide identity. Detailed specimen records and sequence information were uploaded to the BOLD (http://www.boldsystems.org) and can be found within the Working Group 1.4 Initiative "Human Pathogens and Zoonoses" container "MCBCS-Surveillance of mosquitoes and *Culicoides* in the Basque Country, Spain." The digital object identifier (DOI) for publicly available projects in BOLD is doi: dx.doi.org/10.5883/DS-MQBMBC.

IV.1.2.5. Data analysis

Statistical analyses were performed using the R statistical software version 3.6.1 (R Core Team, 2022). Generalised linear models (GLM) were used to compare mosquito catches by sampling strategies (CDC traps *vs*. SN). Multivariate GLM were used to evaluate the associations between culicid abundance (catches/trap/night) related to year (2018 and 2019), months of sampling (July–October, shared period for both years), and aquatic setting (Salburua and Urdaibai). Owing to data over-dispersion, negative binomial generalised linear models (NBGLM) were applied (O'Hara and Kotze, 2010) using the MASS package (Venables and Ripley, 2002). The best model was selected with the "MuMIn" package using the "dredge" function (Barton and Barton, 2020), which is based on the Akaike Information Criterion and corrected to the sample size (AICc). The overall fit of the model was evaluated using the likelihood ratio test to compare the best model with the null model. Species richness (S) and Shannon-Wiener's diversity index (H') were calculated to compare biodiversity among wetlands, using "diversity" function of the R package "vegan". Because not all the specimens were analysed to identify the species within the *An. maculipennis*, *An. claviger*, and *Cx. pipiens* complexes, each complex was considered a species group for the analysis of diversity. Graphical representation of seasonality in mosquito population flight periods were carried out using catches obtained in 2019 using CDC traps, comprising a six-month sampling period (May–October).

IV.1.3. Results

IV.1.3.1. Species composition and abundance

A total of 1,529 adult mosquitoes (254 males and 1,275 females) were collected from the two aquatic environments of Basque Country (northern Spain) using CO_2-baited CDC traps and SN. A total of 32 and 78 CDC trap/night contents were examined in 2018 and 2019, respectively. Twenty-two native mosquito species: six *Culex* spp., five *Culiseta* spp., three *Anopheles* spp., six *Aedes* spp., and two *Coquillettidia* spp. were identified (Table 4). Overall, the most abundant species was *Cq. richiardii* (n = 379, 24.8%), followed by *Ae. rusticus* (n = 220, 14.4%), *Cx. pipiens* s.l. (n = 188, 12.3%), and *Cx. modestus* (n = 143, 9.4%) (Table 4). Species composition varied depending on the aquatic setting and both habitats shared 38.1% of the mosquito species captured by CDC traps. *Aedes rusticus* was the most abundant species in Salburua (43.3% of the total catches) whereas *Cq. richiardii* was the predominant species in Urdaibai (35.3%). Molecular analysis of 104 female mosquitoes generated 94 barcoding COI sequences of 405–514 bp in length with 98.7–100.0% homology when compared with GenBank sequences. A selection of these sequences (n = 87 and 17 species) was deposited in GenBank under accession numbers OQ361883 to OQ361929, and a phylogenetic tree was constructed (Figure 12).

Two species within the *An. maculipennis* complex (*An. atroparvus* in Urdaibai, n = 4 and *An. maculipennis* s.s. in Salburua, n = 2) and three ecoforms of *Cx. pipiens* s.l. (*Cx. pipiens pipiens,* n = 19; *Cx. pipiens molestus,* n = 2; and *Cx. pipiens* hybrids, n = 1) were determined using molecular methods.

IV.1.3.2. Assessment of adult mosquito trapping

CO_2-baited CDC traps collected 1,173 specimens (4.6% males and 95.4% females), whereas netting on vegetation retrieved only 356 specimens (56.2% males and 43.8% females). Although CDC traps collected approximately three times more specimens than the SN, these differences were not significant in the overall mosquito catches ($p = 0.83$) or in the case of species such as *Cq. richiardii, Ae. rusticus or Cx. pipiens* s.l. However, SN was more effective for collecting *Cx. territans* and *Ae. detritus* ($p < 0.001$ and $p < 0.05$, respectively), while CDC-traps were more effective for collecting *Cx. modestus* and *An. claviger* s.l. ($p < 0.01$ and $p < 0.05$, respectively) (Table 4). Furthermore, male mosquito catches were significantly higher for sweep net than CDC traps (U = 1213, $p < 0.001$). Blood-fed mosquitoes (n = 53) were trapped by CDC traps (31.4%) as well as sweeping (68.6%).

Table 4. Adult mosquitoes trapped with CDC traps baited with CO_2 (CDC) and sweep netting (SN) in 2018 and 2019 in the two aquatic settings from the Basque country.

Culicidae species	Salburua				Urdaibai				Total	
	CDC	SN	Total	%	CDC	SN	Total	%	Total	%
Ae. vexans	1	0	1	0.2	0	0	0	0	1	<0.1
Ae. cantans	75	14	89	17.5	0	1	1	0.1	90	5.9
Ae. caspius	0	0	0	0	34	29	63	6.2	63	4.1
Ae. detritus	0	0	0	0	43	68	111	10.9	111	7.3
Ae. rusticus	174	46	220	43.3	0	0	0	0	220	14.4
Ae. sticticus	3	0	3	0.6	0	0	0	0	3	0.2
An. claviger s.l.	46	5	51	10.0	53	5	58	5.7	109	7.1
An. maculipennis s.l.	4	1	5	1.0	6	1	7	0.7	12	0.8
An. plumbeus	0	0	0	0	1	0	1	0.1	1	<0.1
Cq. buxtoni	0	0	0	0	98	18	116	11.4	116	7.6
Cq. richiardii	19	0	19	3.7	253	107	360	35.3	379	24.8
Cs. annulata	15	3	18	3.5	16	5	21	2.1	39	2.6
Cs. fumipennis	2	0	2	0.4	3	0	3	0.3	5	0.3
Cs. litorea	2	1	3	0.6	1	0	1	0.1	4	0.3
Cs. morsitans	0	0	0	0	10	6	16	1.6	16	1.0
Cs. subochrea	1	0	1	0.2	0	0	0	0.0	1	<0.1
Cx. hortensis	0	1	1	0.2	0	0	0	0	1	<0.1
Cx. modestus	1	0	1	0.2	140	2	142	13.9	143	9.4
Cx. pipiens s.l.	71	14	85	16.7	93	10	103	10.1	188	12.3
Cx. territans	0	2	2	0.4	2	7	9	0.9	11	0.7
Cx. theileri	1	2	3	0.6	0	0	0	0	3	0.2
Cx. torrentium	0	0	0	0	1	2	3	0.3	3	0.2
Not identified	1	2	3	0.6	3	3	6	0.6	9	0.6
Total	**416**	**92**	**508**		**757**	**264**	**1021**		**1529**	

IV.1.3.3. Seasonal activity

The flight period activity of the mosquito species extended over the entire sampling season (May–October 2019) (Figure 13). Overall, the population dynamics of most mosquito species showed a single large peak of activity, followed by a small peak in some species. In Salburua, mosquitoes peaked in the first half of June (1st–15th June), driven mainly by the emergence of *Ae. rusticus* and the start of the season of *Ae. cantans* (Figure 13). Following this period, the mosquitoes were active, but at lower emergence levels. In Urdaibai, two peaks (bimodal pattern) were observed, with the highest abundance of mosquitoes occurring in the second half of June (16th–30th June), driven by *Coquillettidia* spp., followed by a minor peak in the second half of August (16th–31st August), driven by *Cx. modestus* (Figure 13).

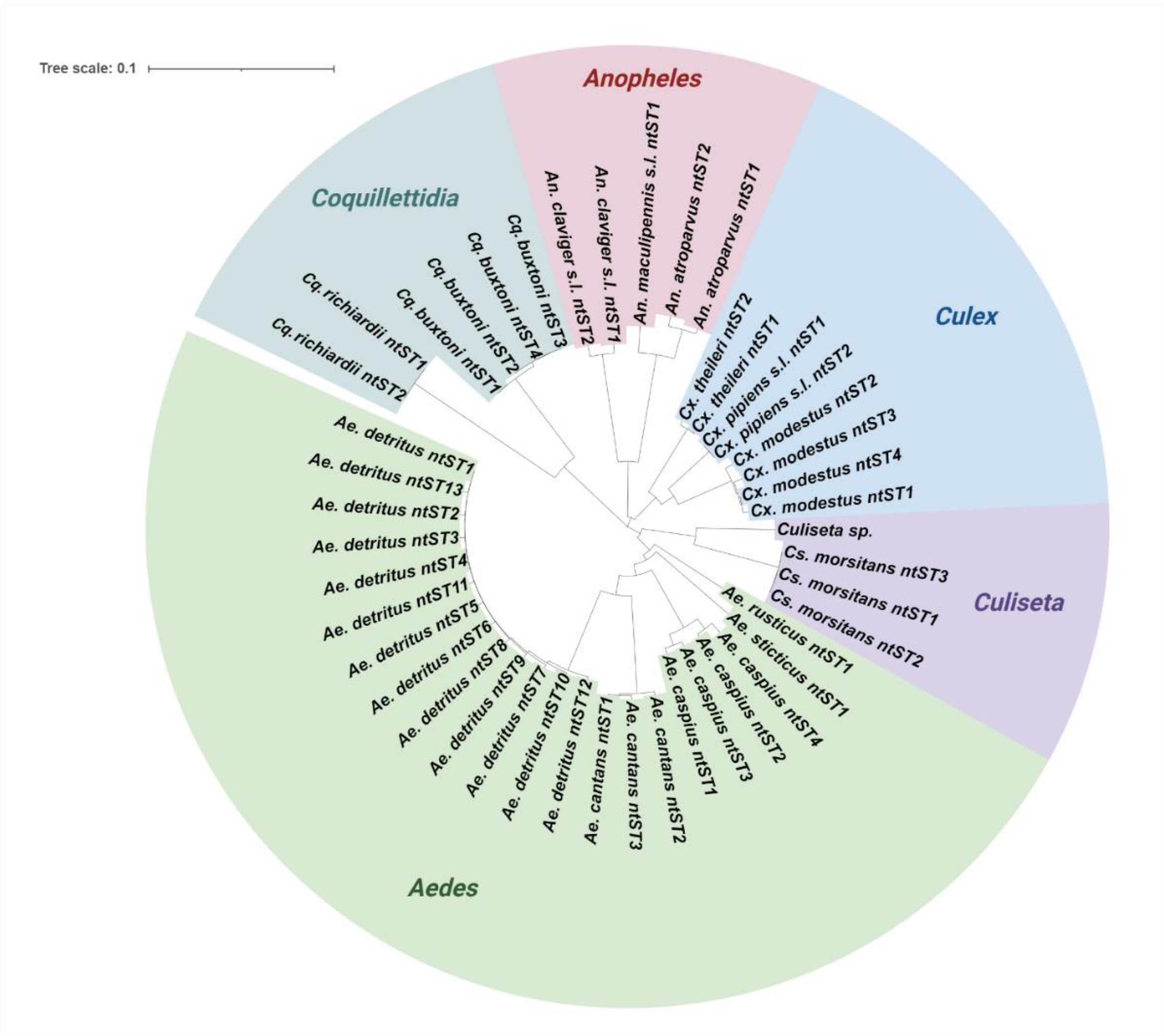

Figure 12. Phylogenetic tree based on the COI gene (378 bp).

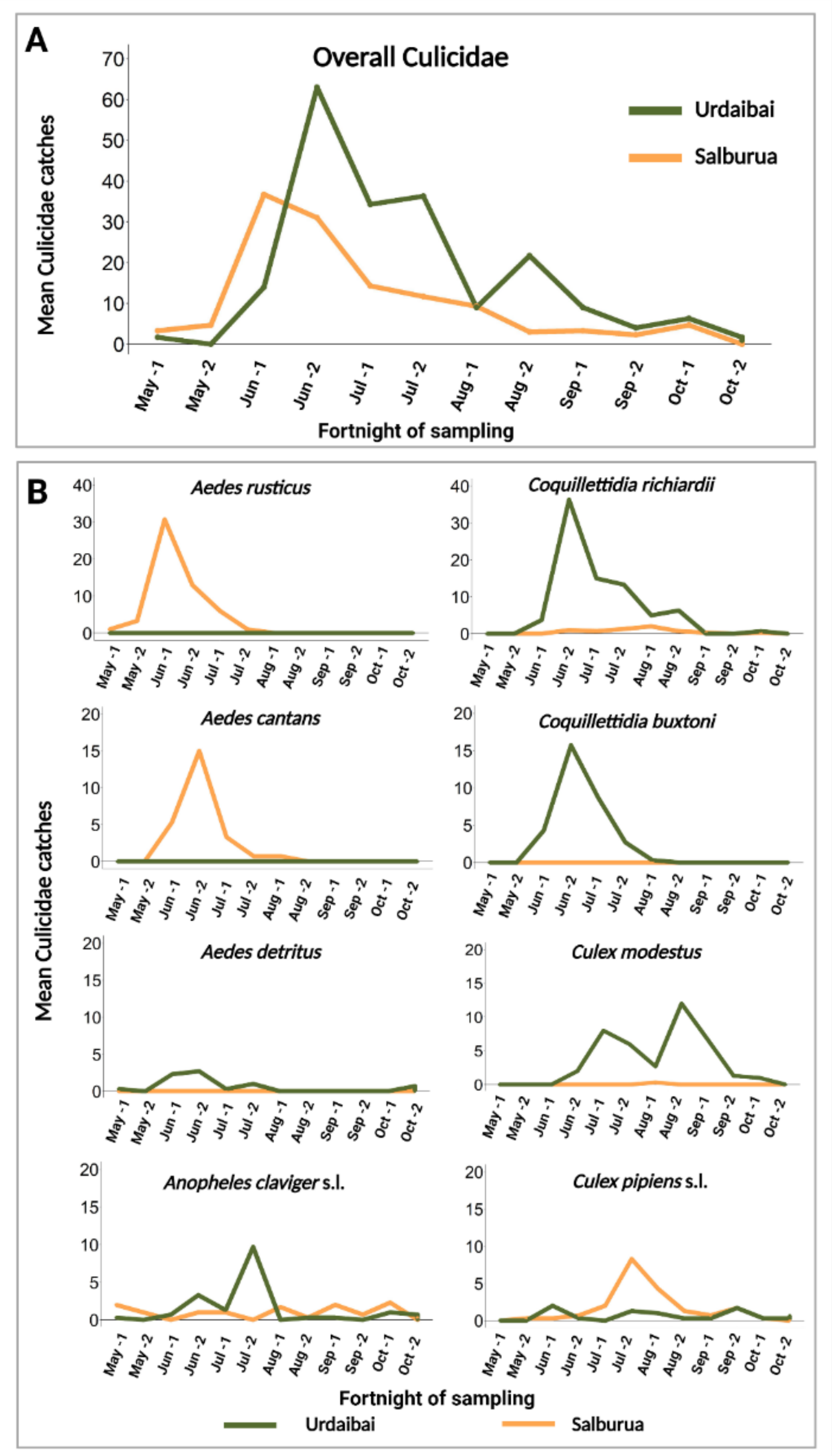

Figure 13. Seasonal flight activity of Culicidae species at both aquatic environments in 2019. (A) overall Culicidae and (B) most abundant species.

IV.1.3.4. Human landing catches (HLC)

A total of 85 host-seeking females of eight species were caught landing at the human operator Table 5). *Aedes rusticus* was the species more commonly collected on humans (n = 46;

54.1% of the total catches), followed by *An. claviger* s.l. (n = 11, 12.9%), *Aedes detritus* (n = 8, 9.4%), and *Aedes cantans* (n = 7, 8.2%). Host-seeking females in Salburua showed a maximum peak in activity in May (n = 25), followed by a progressive decrease during the study period (June, n = 19; July, n = 9; August, 0 catches; September, n = 2; and October, 0 catches). In contrast, host-seeking females in Urdaibai showed consistent activity from May to September (May, n = 4; June, n = 4; July, n = 7; August, n = 4; September, n = 9; and October, n = 1). A higher number of mosquitoes landing on the operator was observed in Salburua (56 catches) than in Urdaibai (29 catches). Some of these anthropophilic mosquito species were not found at the breeding sites (*Ae. rusticus*). Conversely, some mosquito species abundant at the larval sites (*Cx. pipiens* s.l.*, Cx. territans, Cx. hortensis,* and *Cs. annulata*) were not caught landing on the operator (Figure 14; Table 5).

Table 5. Comparison of mosquito catches obtained in 2019 by CO_2 baited CDC traps, sweep netting (SN), human landing (HLC) and captures of larvae by dipping (DI).

Culicidae species	**Salburua**				**Urdaibai**			
	CDC	**SN**	**HLC**	**DI**	**CDC**	**SN**	**HLC**	**DI**
Ae. cantans	75	14	7	0	0	1	0	0
Ae. caspius	0	0	0	0	23	26	1	0
Ae. detritus	0	0	0	0	22	55	8	42
Ae. rusticus	165	33	46	0	0	0	0	0
Ae. sticticus	3	0	0	0	0	0	0	0
Ae. vexans	1	0	0	0	0	0	0	0
An. claviger s.l.	37	5	1	26	53	5	11	18
An. maculipennis s.l.	2	0	0	0	2	0	0	0
An. plumbeus	0	0	0	0	1	0	0	0
Cq. buxtoni	0	0	0	0	95	17	4	0
Cq. richardii	19	0	0	0	241	107	2	11
Cs. annulata	6	3	0	27	13	3	0	11
Cs. fumipennis	2	0	0	0	3	0	0	0
Cs. litorea	2	1	0	0	1	0	0	0
Cs. longiareolata	0	0	0	2	0	0	0	0
Cs. morsitans	0	0	0	0	5	5	0	0
Cs. subochrea	1	0	0	0	0	0	0	0
Cx. hortensis	0	1	0	0	0	0	0	32
Cx. modestus	1	0	0	0	119	1	3	0
Cx. pipiens s.l.	60	4	0	22	25	9	0	31
Cx. territans	0	2	0	53	0	7	0	59
Cx. theileri	0	0	0	0	0	0	0	0
Cx. torrentium	0	0	0	0	1	2	0	0
Not identified	0	3	2	0	2	2	0	0
Total	**374**	**66**	**56**	**130**	**606**	**240**	**29**	**204**

IV.1.3.5. Larval rearing sites

The developmental sites of eight mosquito species (n = 130 in Salburua and n = 204 in Urdaibai) were identified across the nine microhabitats inspected (Table 6). The most abundant species was *Cx. territans* (n = 112), followed by *Cx. pipiens* s.l. (n = 53), and *An. claviger* s.l. (n = 44). Within natural habitats, vast permanent pools were particularly productive to *Cs. annulata, An. claviger,* and *Cx. territans,* whereas *Culex, Anopheles*, and *Aedes* species preferred temporary pools, ephemeral puddles or pools, and ponded ditches or streams, respectively (Table 6). *Coquillettidia richiardii* was successfully collected in *Typha* roots in the marsh but they were unable to reach adult stage (Table 6). Artificial ponds and troughs are prone to host artificial container breeding species such as *Cx. pipiens* s.l., *Cs. longiareolata*, and *Cx. hortensis*. Notably, *Cs. longiareolata* was not captured with CDC traps or by SN in Salburua; however, a few specimens were observed at the larval sites (Figure 14; Table 6). Mosquito immature stages were active during the six-month trapping period; however, the inconsistency of the water levels (flushing and drainage) prevented further analysis.

Table 6. Summary of the mosquito species collected by dipping in both natural and artificial aquatic environments the year 2019 (May-October).

Aquatic ecosystem	**Species**	**Type of breeding site [1]**									**Total**
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	
Salburua	*An. claviger s.l.*	3	0	0	9	16	-	-	-	-	28
	Cs. annulata	0	0	14	5	8	-	-	-	-	27
	Cs. longiareolata	0	0	2	0	0	-	-	-	-	2
	Cx. pipiens s.l.	0	8	11	1	2	-	-	-	-	22
	Cx. territans	0	0	26	10	15	-	-	-	-	51
Urdaibai	*Ae. detritus*	-	-	-	-	-	22	24	0	0	46
	An. claviger s.l.	-	-	-	-	-	15	1	0	0	16
	Cq. richiardii *	-	-	-	-	-	0	11	0	0	11
	Cs. annulata	-	-	-	-	-	11	0	0	0	11
	Cx. hortensis	-	-	-	-	-	0	0	0	28	28
	Cx. pipiens s.l.	-	-	-	-	-	3	28	0	0	31
	Cx. territans	-	-	-	-	-	41	17	3	0	61
	Total	**3**	**8**	**53**	**25**	**41**	**92**	**81**	**3**	**28**	**334**

[1] Sites A-E = (A) ditch, (B) artificial pond, (C) puddle, (D) forest temporary pool, (E) river edge. Sites F-I = (F) diverse pools (shallow pools in the woodland and pools with vegetated margins in open pastures) (G) natural shallow channels of water (maximum width and depth 2 m and 0.4 m, respectively), (H) open-marsh with *Typha* sp., (I) artificial container. * Death larvae were also counted.

IV.1.3.6. Analysis of diversity and variables affecting mosquito abundance

The H' value for Salburua was 1.83 and for Urdaibai was 2.09, while the S for both sites was 17. Multivariate models revealed that total mosquito abundance was positively associated with the water ecosystem, the total abundance of mosquitoes in the marsh of Urdaibai being significantly higher than that in the wetland of Salburua (Table 7). Moreover, the number of mosquito catches was positively associated with the sampling period, with high catches at the beginning of the summer and decreasing progressively during the following months (Table 7).

Table 7. Summary of best negative binomial regression models for total number of Culicidae per trap and night. The variable "year of sampling" was not selected for the best model.

Variables	**Abundance per trap/night**		
	Est ± SE [1]	**z** [2]	**p-value** [3]
Water setting			
Salburua	Ref. [4]		
Urdaibai	1.10 ± 0.26	4.24	< 0.001
Month of sampling			
July	Ref.		
August	-0.81 ± 0.33	-2.40	0.016
September	-1.43 ± 0.35	-4.09	< 0.001
October	-1.98 ± 0.36	-5.52	< 0.001

[1] Est ± SE = Estimate ± Standard Error; [2] z = statistic z-value; [3] p = p-value; [4] Ref. = reference category

IV.1.3.1. Host DNA blood-meals

Among the 53 female mosquitoes analysed (39 blood-fed and 14 gravid females), 47 yielded a COI DNA barcode sequence, and host DNA sequences were retrieved from 32 mosquito specimens (60.4% yield). Thus, high success in host DNA identification was recorded in blood-fed female mosquitoes, regardless of their blood digestion status [Sella scale 2: 15/15 (100%); scale 3: 2/3 (67%); scale 4: 3/4 (75%); scale 5: 3/4 (75%); and scale 6: 6/13 (46%)]. The host DNA was successfully identified in only two of the 14 (14%) early gravid specimens. Blood-fed mosquitoes were trapped in May (n = 1), June (n = 12), July (n = 8), August (n = 17), September (n = 5), and October (n = 10).

Figure 14. Relative abundance of different mosquito species trapped with the four sampling methods during 2019 in both aquatic ecosystems. CDC = CDC light traps baited with CO_2; SN = Sweep netting; HLC = Human landing catches; DI = Dipping.

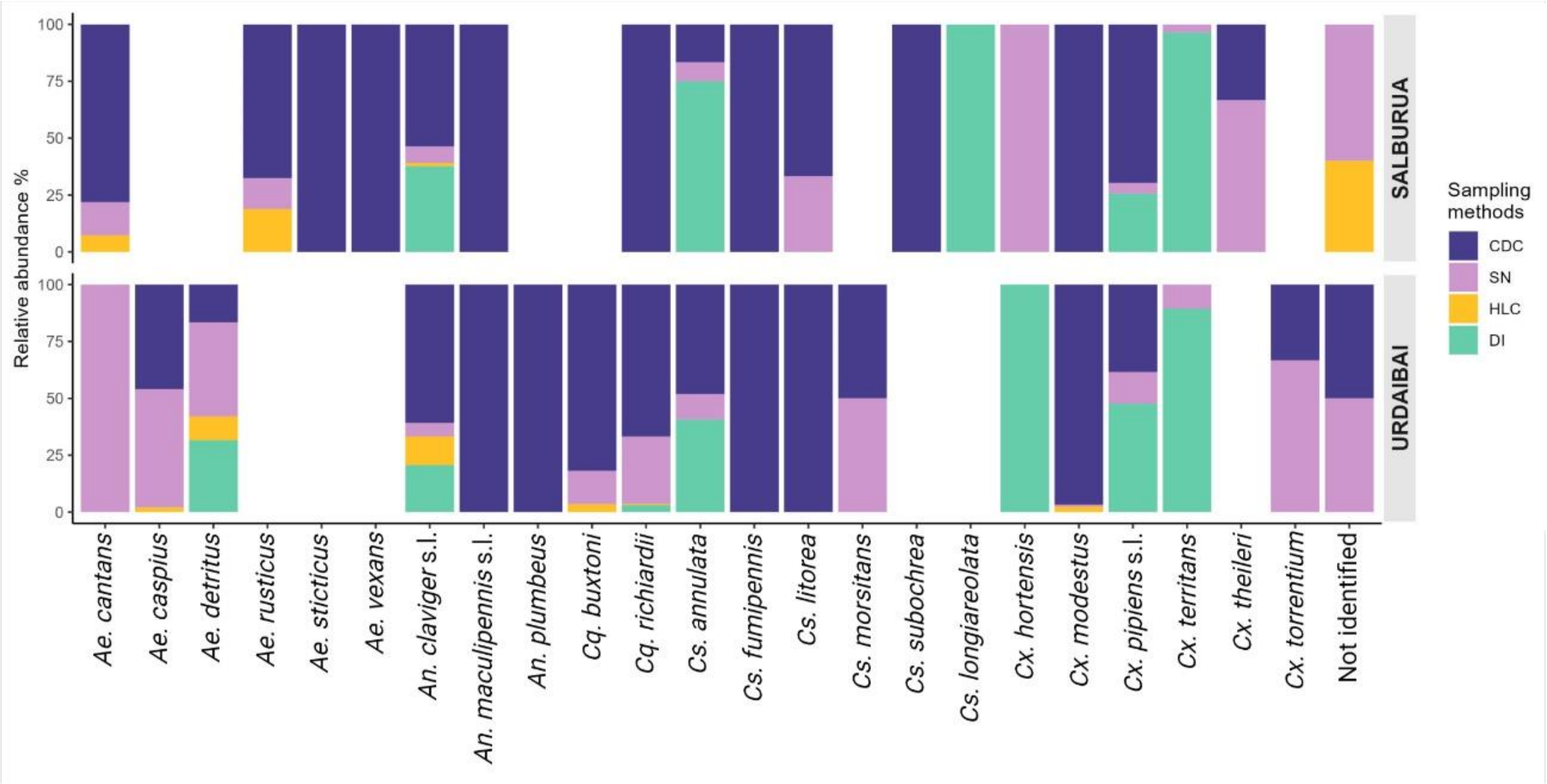

Table 8. Host DNA blood-meals determined in mosquito species from two water ecosystems investigated in 2018 and 2019.

Mosquito species	**Salburua**		**Urdaibai**	
	No.[1]	**Host DNA**	**No.**	**Host DNA**
Aedes cantans	1	*Cervus elaphus* (1) [2]		
Aedes rusticus	12	*Cervus elaphus* (8)		
Aedes caspius			3	*Sus scrofa* (1)
Aedes detritus			9	*Bos taurus* (6), *Ovis aries* (2)
Anopheles claviger s.l.	1	*Meleagris gallopavo* (1)	2	--
Coquillettidia buxtoni			5	*Sus scrofa* (1)
Coquillettidia richiardii			3	*Equus caballus* (1), *Sus scrofa* (1)
Culex pipiens s.l.	2	*Parus major* (1)	1	*Turdus philomelos* (1)
Culex modestus			3	*Anas platyrhynchos* (1), *Turdus* sp. (1), *Homo sapiens* (1)
Culiseta annulata	2	*Meleagris gallopavo* (2)	6	*Bos taurus* (1)
Culiseta litorea			1	--
Culiseta morsitans			2	*Turdus merula* (1)

[1] number of mosquitoes analyzed; [2] in parenthesis the number of mosquitoes in which host DNA was identified.

At least 11 different vertebrate host species, including six mammals and five birds, were identified from 11 mosquito species (Table 8). *Aedes* spp. (n = 18) and *Coquillettidia* spp. (n = 3) fed on ungulates, whereas *Culiseta* spp., *Culex* spp., and *An. claviger* s.l. fed primarily on birds (Table 8). A distinct host-feeding pattern was observed depending on the aquatic setting; mosquitoes fed predominantly on red deer and birds in Salburua, whereas they fed on a great variety of domestic (horses, cattle, and sheep) and wild ungulates (wild boar) as well as birds and humans in Urdaibai.

IV.1.4. Discussion

As a critical component of disease surveillance programs and ecosystem health assessments, the mosquito community composition and feeding preferences in two crucial stopovers for migratory birds in the Basque Country have been studied. Different collection methods are required to obtain a better representation of mosquito species composition in a given area (ECDC, 2018). Therefore, this- study employed a multi-trapping approach (CO_2-baited CDC traps, SN, HLC, and larvae collection) to obtain comprehensive information on mosquito fauna. A wide set of mosquito species was found to be in line with what is expected in natural areas compared to urbanised areas (Wilke et al., 2021a).

The collection of flying mosquitoes using baited traps is less time-consuming than other trapping methods; however, different traps vary in their ability to catch certain species. Since some species move long distances from their larval sites and others are difficult to be collected using adult suction traps, the direct collection of immature stages in aquatic habitats should be pursued (Wilke et al., 2022). In this study, *Cx. hortensis, Cx. territans, Cs. annulata,* and *Cs. longiareolata* were collected at immature stages, but were scarce or absent in the CDC catches. In contrast, larvae of several mosquito species were not found in the breeding sites examined, even though adult mosquitoes were trapped using CDC traps and HLC (*Ae. rusticus, Cx. modestus,* and *Cq. buxtoni*). The reasons of these contradictory findings could be found in the biases of CDC traps, the dispersal patterns of emerging adults, or their resting behaviour, among others (ECDC, 2014). In addition, the complementary technique of SN is a valuable tool for collecting blood-fed and gravid mosquitoes, males, or species not attracted by CDC traps (ECDC, 2018), as observed in this study with *Cx. territans* and *Ae. detritus*. These observations highlight the importance of using several techniques simultaneously to accurately characterise and define mosquito biodiversity in specific habitats, which is critical to achieving reliable and actionable results (Reisen et al., 1999).

The affinity of mosquito species to bite humans was evaluated in both aquatic areas using opportunistic HLC, which is considered the gold standard approach for the collection of anthropophilic mosquitoes. In this study, eight species were recorded biting the human collector, although these numbers might be increased by trapping over the crepuscular period (Becker et al., 2020; Brugman et al., 2017a), for example, *Culex* species bite predominantly at dusk (Hawkes et al., 2020). In Salburua, *Ae. rusticus* was the most common species collected on both humans and suction traps particularly at the end of the spring season (May–June). This species was responsible for the mosquito biting complaints, which caused the hospitalisation of two people working in Salburua Park (Oier Quesada, personal communication). In Italy, *Ae. rusticus* is a problematic species that causes severe biting in local tourism in the Toscana region (Mosquitoweb Difenditi Dalle Zanzare, 2022). In Urdaibai, several mosquito species (*An. claviger* s.l., *Ae. detritus, Cq. richiardii, Cq. buxtoni*, and *Cx. modestus*) showed some degree of propensity to bite humans, which is in accordance with other studies conducted in Britain (Brugman et al., 2017a; Hawkes et al., 2020). Other mosquito species (*Cx. pipiens* s.l., *Cx. territans*, *Cx. hortensis,* and *Cs. annulata*) were not collected on humans possibly as they are typically crepuscular, ornithophilic or do not disperse far from larval sites.

The study determined the larval habitats of eight mosquito species. The most common species found breeding was *Cx. territans* (33.5%), followed by *Cx. pipens* s.l. (16.2%), and *An.*

claviger s.l. (13.2%). Most species were retrieved from ditches, puddles, and small pools; however, large water pools were considerably unproductive to harbour immature stages of mosquito species, except those of *Cx. territans* that are commonly trapped in open pools of water holdings (Bueno-Marí, 2010). Notably, larval sites of monocyclic *Ae. rusticus* were not spotted even though their typical larval sites, including woodland pools and ditches of various origins with vegetation, were inspected (Grego and Zamburlini, 2021). Mosquito species such as *Cx. modestus, An. atroparvus, Ae. detritus*, and *Ae. caspius*, which usually breed in brackish and saline water (Hawkes et al., 2020; Medlock and Vaux, 2015a; Roiz et al., 2015), were exclusively present in the marsh, whereas other species linked to temporary freshwater pools in wet woodlands, such as *Ae. rusticus* and *Ae. cantans* (Hawkes et al., 2020; Medlock and Vaux, 2015a) were present in the wetland. *Coquillettidia richiardii*, the most abundant species identified in this study, is a relatively common species that breeds in permanent water with abundant vegetation (Hawkes et al., 2020); however, its congeneric *Cq. buxtoni* seems to have a limited range distribution in Europe (Becker et al., 2020; Martínez-Barciela et al., 2021), and in this study they were spotted only in the marsh. Pre-imaginal stages of *Coquillettidia* spp. are not easily detected (Medlock and Vaux, 2015b) unless aquatic plant stems and root systems are removed and examined (Johnson & Russell, 2019), which explains the low records of these species in other studies. Notably, species frequently observed in Mediterranean wetlands in southwestern Spain (Roiz et al., 2015, 2012a), such as *Culex theileri* and *Culex perexiguus*, are rare or absent in the two aquatic systems studied in northern Spain and other Atlantic climatic regions (Hawkes et al., 2020). In contrast, other species with high ecological plasticity, such as *Cx. pipiens* s.l. were found in both habitats, as in other studies (Joyce et al., 2018).

The study of mosquito population dynamics is important in understanding the periods of activity of each species, with the aim of predicting biting risk for humans and animals. In the present study, the earliest species that emerged in spring, after the temperatures in autumn decreased and the cold winter period was set in, was *Ae. rusticus* (Becker et al., 2020). This snowmelt species showed a well-defined unimodal peak in activity at the beginning of June, as previously reported (Becker et al., 2020). Other univoltine species, including *Ae. cantans*, *Cq. richiardii*, and *Cq. buxtoni,* peaked at the end of June, whereas other culicids such as *Cx. pipiens* s.l., *Cx. modestus*, *An. claviger* s.l., and *Ae. detritus* showed a bivoltine or multivoltine pattern (Medlock and Vaux, 2015b).

The differences in diversity and mosquito abundance between the two aquatic systems could be attributed to abiotic and biotic factors. The Salburua wetland, which is located in the transition zone between Atlantic and Continental Mediterranean climates, has more extreme climatic conditions than Urdaibai, which is located on the coast and is influenced by the mild Atlantic climate. During the summer, unlike Urdaibai, the Salburua wetland dries up completely; therefore, precipitation (together with relative humidity and average temperatures) considerably explains the variations in mosquito abundance. In contrast, in Urdaibai, other factors may have more weight in explaining the differences in the abundance of mosquitoes. For example, the level of the sea tide is associated with the emergence and abundance of species such as *Ae. detritus* and *Ae. caspius* that benefit from the high tides that flood the salt marshes and create larval breeding sites (Roiz et al., 2014). Regarding biotic factors (not included in this study), the aquatic community of predators that feed on mosquito larvae is reported to modulate mosquito abundance (Shaalan and Canyon, 2009). In addition, the capacity of wetlands to retain water during the year (permanent or temporary wetlands) determines the wildlife species inhabiting them (Wellborn et al., 1996).

The feeding habits of mosquitoes are critical factors in the transmission of vector-borne pathogens (Montgomery et al., 2011; Yan et al., 2021). Some species feed opportunistically on a wide range of hosts, whereas others feed on a limited range of hosts (Gibson and Torr, 1999). Owing to the low number of blood-fed specimens recorded in our study, it is difficult to establish more robust conclusions. Nonetheless, the host DNA from mosquito blood meals reflects the vertebrate species present in each aquatic ecosystem. *Aedes* spp. and *Coquillettidia* spp. primarily fed on ungulates, whereas *Culex* and *Culiseta* spp. fed on birds and mammals. The feeding habits of both genera showed strong ornithophilic preferences along an urban-natural gradient near the region of this study. Humans were uncommon hosts, and only a single specimen of *Cx. modestus* contained human blood, despite the relatively high frequency of walkers in both natural settings. Considering that *Cx. modestus* is a major human-biting mosquito species recorded as a WNV bridge vector in wetlands in France (Ponçon et al., 2007), it would be interesting to assess whether the population of *Cx. modestus* present in these aquatic environments harbours this virus.

Several other mosquito species found in the study area have been described as potential vectors of the WNV (*Culex* spp.), Sindbis virus (*Culex* spp.), Usutu virus (*Cx. torrentium*, *Cx. pipiens* s.l., and *An. maculipennis* s.l.), Tahyna virus (*Ae. vexans, Cs. annulata*, and *Ae. cantans*), protozoa causing malaria (*Anopheles* spp.), and filarioid nematodes (*Culex* spp. and *Ae. vexans*) (Becker et al., 2020; Calzolari et al., 2013; Hawkes et al., 2020; Medlock and Vaux, 2015a; Schaffner et al., 2001). However, other species such as *An. claviger* s.l., *Ae. rusticus, Coquillettidia* spp., and *Culiseta* spp. do not pose a concern to human health. However, the feeding behaviour of some of these species on humans or birds indicates that they are putative bridge vectors of arboviruses (Hawkes et al., 2020; Wang et al., 2021).

The interaction of mosquitoes with birds and wild animals is potentially frequent in these aquatic ecosystems, a condition that favours WNV emergence and amplification. Thus, considering that both aquatic systems are located in one of the major migratory routes between Europe and Africa, and wild birds are able to carry and introduce WNV over large distances into new areas, there is a need to reinforce surveillance and early detection of WNV in animals (Jourdain et al., 2007). Therefore, the information provided here might be critical as some mosquito species can act as bridge vectors between birds and humans.

This study highlights the importance of studying mosquito diversity. Mosquito sampling strategies that use techniques complementary to ordinary adult mosquito surveillance systems can potentially supplement the sampling power of the surveillance programs. The data obtained here provided local information on the ecology of several mosquito species, such as their biting behaviour, choice of resting sites, aquatic habitats, and the highest relative abundance levels of immature mosquito stages. Based on the data recorded, and compared to the wetland of Salburua, Urdaibai marsh showed more abundance of mosquitoes and more species regarded as potential vectors of pathogens. The results presented here can be used by local health authorities to design the best management and sanitary strategies for mosquito control operations, because the socio-economic relevance of mosquito control requires considering nuisance, vector-borne diseases, and environmental effects together.

Study 2: Mosquitoes in rural and peri-urban areas with presence of animals

IV.2.1. Background

Blood-feeding arthropods are annoying biters as well as vectors of many pathogens which affect humans and animals (Lehane, 2005). Vector-borne diseases are a group of globally distributed and rapidly spreading illnesses, and although tropical areas are more exposed, Europe is currently experiencing an increase in the number of human vector-borne diseases and the discomfort inflicted by bites. Mosquitoes can transmit several imported and indigenous diseases (Calzolari, 2016). For example, WNV outbreaks exhibited a sharp increase in Central Europe in 2018 (Burki, 2018). Vector-borne diseases also have an impact on the welfare of pets. Canine and feline vector-borne diseases are caused by a wide range of primarily zoonotic pathogens, including viruses, bacteria, protozoa, and helminths (Baneth et al., 2016), which emphasizes the importance of pets as reservoirs. These pathogens are transmitted by a variety of vectors, such as ticks, fleas, mosquitoes, and phlebotomine sand flies, among others (Otranto and Dantas-Torres, 2010). Although research on canine and feline vector-borne infections is steadily increasing, the vectors of many vector-borne pathogens detected in animals are yet unknown (Baneth et al., 2016).

Animal protection centers (APCs) are non-profit organizations that rescue, care, and provide protection, assistance, and shelter to animals, mostly dogs and cats. Every year, approximately 131,000 dogs and cats arrive at the community APCs in Spain (Fundación Affinity, 2018), and almost 50% of them are adopted by citizens (Fundación Affinity, 2018). This intense flow favours contact between humans (APC workers and visitors) and companion animals, which increases the risk of transmission of arthropod-borne zoonotic diseases whenever the competent vectors are present. In Spain, as in many other European countries, there is not much information about the mosquitoes associated to dog and cat shelters. Since many of these APCs are in periurban areas, vector species found at these settings could be used as a proxy of the vector communities present in urban areas. Therefore, to better understand the diversity of mosquitoes in these APCs, the present work was aimed at: (i) studying the diversity and abundance of Culicidae, and (ii) analysing the association of catches with various variables (type of light, bait, location, and month of sampling).

IV.2.2. Material and Methods

IV.2.2.1. Study area

The study was conducted on three municipal APCs located in the vicinity of the three provincial capitals (APC1, Vitoria-Gasteiz - Araba; APC2, Bilbao - Bizkaia; and APC3, Donostia-San

Sebastián - Gipuzkoa) of the Basque Country. The Basque Country region covers *ca*. 7,200 Km^2 and has *ca.* 2,188,000 inhabitants. The mountainous terrain defines distinctive climate areas. APC2 and APC3 are located in the green northern region, influenced by an oceanic climate with wet weather and mild temperatures all year round, while APC1 is located in the central-southern area of the Basque Country, influenced by a continental climate with cold winters and warm and dry summers.

The three APCs showed distinctive environmental characteristics, pet census and geographical locations (Figure 15). Whereas APC1 (42.832882, -2.711116) and APC2 (43.273048, -2.908081) were in periurban areas, APC3 (43.294444, -2.065416) was located in a rural area. All three APCs were erected on concrete floor with limits fenced by open-metal panels allowing the free entrance of external insects. The arrangement of the shelters consisted on multiple-shed open row dog kennels/roof shelters style (5-10 dogs per kennel) and outdoor cat houses/huts (5-10 cats per house) or indoor rooms (*ca.* 5-30 cats). Other facilities within the premises included green yards, vegetation patches and veterinary practice areas. The surroundings varied among studied sites, *e.g.* APC1 was surrounded mainly by green areas and field crops, whereas APC2 and APC3 were bordered by woodlands (Figure 15).

IV.2.2.2. Collection methods

CDC (Centers for Disease Control) miniature light traps (John W. Hock Company, FL, USA) were used for the collection of insects. Four CDC light traps were deployed in each APC, two of them (model 512) equipped with standard incandescent bulbs powered with 6V cells, and the other two (model 1212) fitted with ultraviolet (UV) fluorescent lamps connected to 220V. Two of the CDC traps (one incandescent and one UV) were alternatively baited with CO_2 for each sampling. Roughly 1.2 Kg of dry ice were added into drilled-polyethylene boxes and suspended above the traps to deliver a continuous plume of CO_2 during the full length of the sampling. Trapping took place between July 1 and October 31, 2018. A total of eight samplings (one sampling every 15 days) were undertaken at each APC. Traps were set up early in the morning and collected 24 hours later. The location of the traps followed the suggestions of the local staff and was conditioned by the availability of an energy supply for the UV-traps. A distance of at least 10 meters between traps was left in order to minimize, as much as possible, the interaction between light traps.

IV.2.2.3. Mosquito identification

At the laboratory, collected insects were separated into different blood-feeding groups. Male mosquitoes were preserved in 70% ethanol at -20ºC, whereas female mosquitoes were temporally stored in small vials until identification by taxonomic keys (Becker et al., 2020). Adult male mosquitoes were morphologically identified according to the genitalia, using the appropriate keys (Becker et al., 2020).

Female mosquito specimens with doubtful morphological identification were analysed using molecular methods. After DNA extraction by QIAamp DNA Mini kit (Qiagen, Hilden, Germany), a PCR targeting the COI gene was carried out following procedures already described (Simon et al., 1994; Delgado-Serra et al., 2021). PCR amplicons were purified using ExoSAP-IT (Applied Biosystems, Thermo Fisher Scientific, Vilnius, Lithuania) and submitted for Sanger sequencing (Eurofins Genomics, Germany). The sequences obtained were analysed using BioEdit software (v.7.2.5) (Hall, 1999), and compared with the GenBank database by nucleotide sequence homology searches made at the network server of the National Center for Biotechnology Information (NCBI) using BLAST.

To further identify specimens belonging to the *An. maculipennis* complex, a PCR-RFLP analysis of the ITS2 region of the rDNA was done (Vicente et al., 2011). Moreover, identification of the different *Cx. pipiens* forms (*Cx. pipiens pipiens, Cx. pipiens molestus* and its hybrid) was also performed by PCR amplification of the flanking region of the CQ11 microsatellite, following the protocol described by (Bahnck and Fonseca, 2006).

IV.2.2.4. Data analysis

Statistical analyses were performed using R statistical software version 3.6.1 (R Core Team, 2022). Associations between the total abundance of mosquitoes (catches per trap/night) and different variables, such as the type of light (UV and incandescent light), bait (CO_2/non-CO_2), location (APC1, APC2, and APC3), and month of sampling (July, August, September, and October) were analysed. Due to the overdispersion of sample distribution, negative binomial generalized linear models (NBGLMs) were applied (O'Hara and Kotze, 2010) using the MASS package (Venables and Ripley, 2002). The model used log-link distribution to estimate the incidence rate ratio (IRR and their 95% CI confidence intervals) of the dependent variable (number of specimens captured) for each category within each independent variable considered. Multivariate models were constructed with those variables that showed $P < 0.20$ in the univariate analyses. Variables with P < 0.05 in the multivariate models were retained. The possibility of interaction between different variables was examined by constructing different negative binomial regression models compared by using likelihood ratio test (LRT) and Akaike's information criterion (AIC). Species richness (S) and/or Shannon-Wiener's diversity (H´) were calculated to compare biodiversity among APCs and/or between the type of traps.

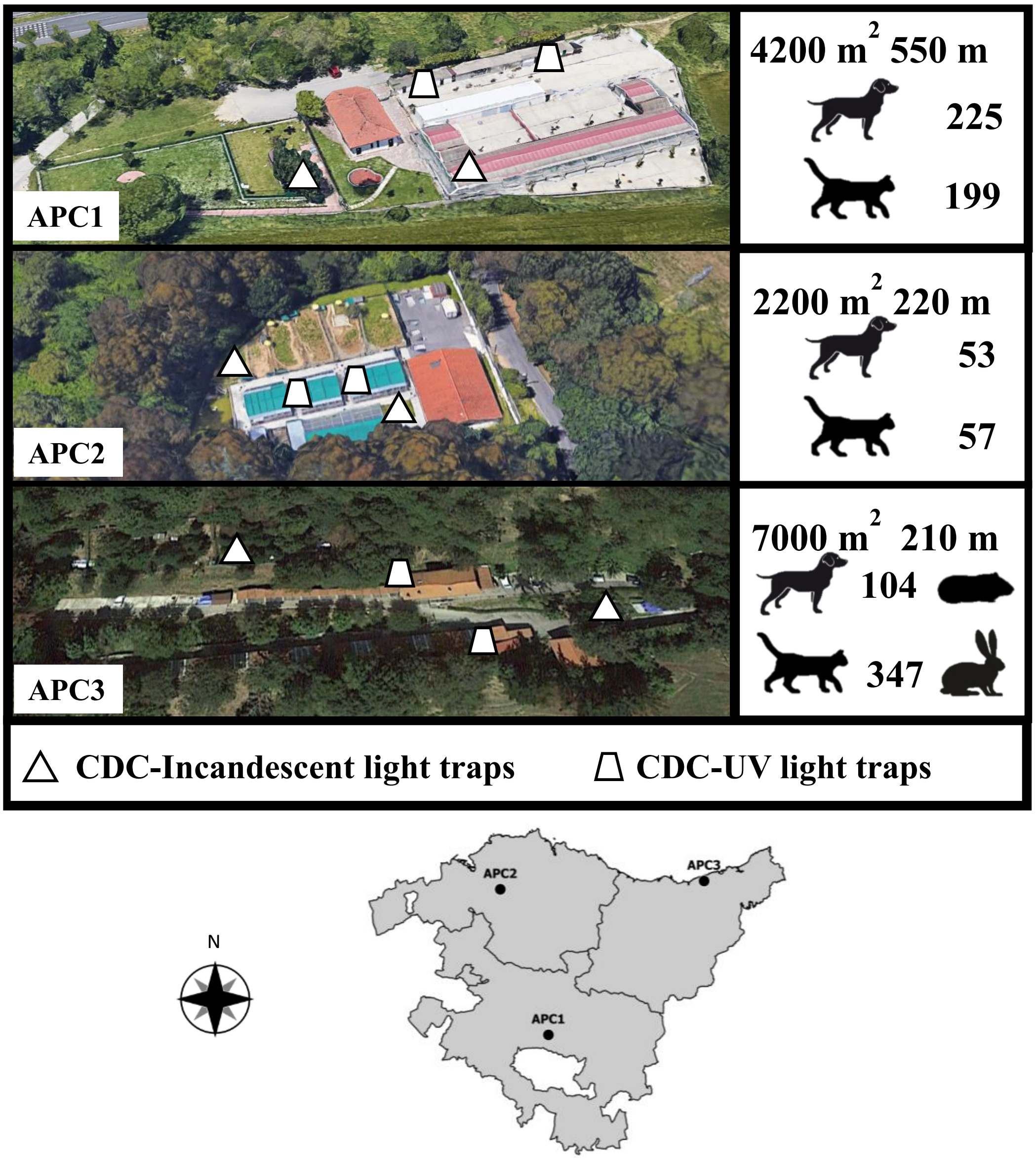

Figure 15. Animal Protection Centers (APC) studied in the Basque Country.
Type of CDC-traps and position in each APC are indicated with symbols (triangles and trapezoids); on the right, the surface area of each APC, altitude, average number of dogs and cats present at sampling and other animal species (if present), are indicated. On the bottom, map of the Basque Country indicating the location of the three studied APCs.

IV.2.3. Results

IV.2.3.1. Abundance and species composition

A total of 224 mosquitoes were collected in the three studied APCs. Eight mosquito species were collected with *Cx. pipiens* s.l./*Cx. torrentium* and *Cs. longiareolata* comprising 76.8% and 18.8% of the total collections, respectively, within the Culicidae family (Table 9). Sequencing analysis of COI gene amplicons confirmed the identification of 27 mosquito female specimens with doubtful morphological identification. They belonged to four species: 16 females from APC1 (1 *Cs. longiareolata*, 1 *An. maculipennis* complex, 12 *Cx. pipiens* s.l. and 2 *Cx. torrentium*), 6 specimens from APC2 (5 *Cx. pipiens* s.l. and 1 *Cx. torrentium*), and 5 specimens from APC3 (1 *Cs. Longiareolata* and 4 *Cx. pipiens*). Sequences shared 98.5-99.8% homology in the 405-514 bp of the COI gene compared. One sequence for each species was submitted to the GenBank database [accession numbers MT506468 (*Cs. longiareolata*): MT506469 (*An. maculipennis* s.l.); MT506470 (*Cx. torrentium*); MT506472 (*Cx. pipiens* s.l.)].

PCR-RFLP analyses allowed the identification of specimens belonging to the *An. maculipennis* complex as *An. maculipennis* s.s. Moreover, the CQ11 microsatellite analysis determined that *Cx. pipiens* s.l. collected in APC1 and APC3 were all *Cx. pipiens pipiens* forms, whereas four *Cx. pipiens* specimens in APC2 were *Cx. pipiens pipiens* (3) and *Cx. pipiens molestus* (1) (Table 9).

Table 9. Species richness and abundance of mosquitoes (Diptera: Culicidae) trapped with CDC-traps in the three Animal Protection Centres (APC).

Mosquito species	**APC1**				**APC2**				**APC3**				**Total**			
	♀	♂	T[1]	%	♀	♂	T	%	♀	♂	T	%	♀	♂	T	%
Cx. pipiens/ Cx. torrentium *	76	10	86	74.8	61	0	61	76.3	23	0	23	79.3	162	10	172	76.8
Cs. longiareolata	18	5	23	20.0	13	1	14	17.5	3	2	5	17.2	34	8	42	18.8
Cs. annulata	1	0	1	0.9	0	0	0	0.0	0	0	0	0.0	1	0	1	0.4
An. plumbeus	0	0	0	0.0	5	0	5	6.3	0	0	0	0.0	5	0	5	2.2
An. maculipennis s.s.	2	0	2	1.7	0	0	0	0.0	0	0	0	0.0	2	0	2	0.9
An. claviger	0	0	0	0.0	0	0	0	0.0	0	1	1	3.4	0	1	1	0.4
Co. richiardii	1	0	1	0.9	0	0	0	0.0	0	0	0	0.0	1	0	1	0.4
Total	**100**	**15**	**115**		**79**	**1**	**80**		**26**	**3**	**29**		**205**	**19**	**224**	
Species richness		**6**				**4**				**3**				**8**		
Shannon-Wiener's diversity		**0.78**				**0.74**				**0.60**				**0.78**		

* COI gene sequencing allowed the identification of three *Cx. torrentium* females (morphologically indistinguishable from *Cx. pipiens*). All males belonged to *Cx. pipiens* species; [1] T=total

The highest species richness and diversity was observed in APC1 (S = 6; H´ = 0.78) whereas the APC3 showed the lowest biodiversity values (S = 3; H´ = 0.60) (().

Traps fitted with UV-light collected 6 species of mosquitoes, whereas incandescent light traps yielded 4 species. Traps equipped with CO_2 collected a few more species (8 species) compared to traps without CO_2 (5).

IV.2.3.2. Population dynamics

Adult mosquitoes exhibited two main peaks (early July and late August) but were present in substantial numbers throughout the end of the sampling period (Figure 16).

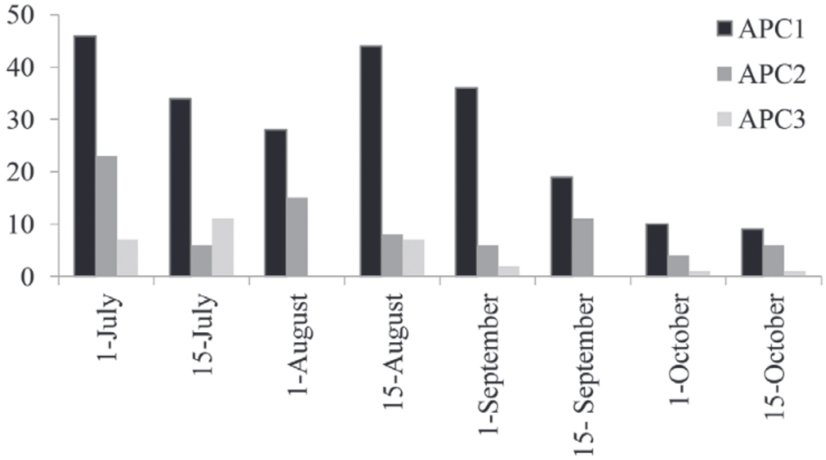

Figure 16. Total abundance of mosquitoes collected in Animal Protection Centers (APC).

IV.2.3.3. Analysis of variables affecting catches of mosquitoes

Univariate analyses revealed statistically significant associations between total abundance of mosquitoes with the type of trap (UV, incandescent light), location (APC1, APC2 and APC3) and month of sampling (July, August, September and October).

Mosquitoes' total abundances were positively associated with the type of trap, location, and month of sampling in the multivariate model (Table 10). Mosquitoes were captured in significant higher numbers at APC1 and APC2 than APC3 (. Month of samplings affected trapping of mosquitoes with the highest captures in July. In August and September also significant IRRs were found with respect to October. Finally, UV-light yielded more mosquitoes than the incandescent light (Table 10).

Table 10. Association of type of traps, location, and month of sampling with the total number of mosquitoes captured (negative binomial regression model).

Variables	Culicidae	
	IRR (CI 95%)	p-value
Location		
APC1	4.46 (2.50-8.12)	<0.001
APC2	3.30 (1.82-6.11)	<0.001
APC3	Ref.	
Month of sampling		
October	Ref.	
July	3.91 (1.99-7.89)	<0.001
August	2.81 (1.42-5.67)	0.003
September	2.40 (1.20-4.88)	0.013
Type of trap		
Incandescent Light	Ref.	
UV-Light	2.18 (1.39-3.45)	<0.001

IRR: Incidence Rate Ratio; CI95%: 95% confidence interval; Ref.: Reference category

IV.2.4. Discussion

This study shows the species composition and abundance of mosquitoes in three APCs from the Basque Country, demonstrating that diverse species were active in pet kennels during the sampling period. Overall, the species richness and abundance of mosquitoes recorded was apparently low, but as far as we know, no comparable data have been published yet. The low diversity might be explained by the relative lack of breeding sites near the sampling sites, as traps were suspended over concrete kennels without noticeable water accumulation (for mosquitoes breeding). In addition, dog and cat kennels were daily cleaned (by water jet spraying) by the APC staff, which might have also contributed to some extent to the limited number of captures.

The efficiency of black/UV-light traps for the collection of mosquitoes was only 2-times higher compared to incandescent light traps, in agreement with previous studies carried out in the same region (González et al., 2016). From these results, we can conclude that traps equipped with UV-light sources are adequate for the surveillance and monitoring of adult mosquitoes. Baited-CO_2 traps collected roughly the same numbers of mosquitoes compared to unbaited traps. This was surprising considering that CO_2 enhances the catches, and it is worldwide reported to be one of the most important stimuli for the orientation of a wide range of blood-feeding arthropods towards their hosts (Guerenstein and Hildebrand, 2008). In mosquitoes, CO_2 has been used for decades in association with suction traps as a routine tool for adult mosquito trapping in entomological studies since it is thought to enhance the capture of host-seeking female of a broad range of mosquito species (Gillies, 1980). However, a possible explanation for the apparent lack of effect of CO_2 in mosquito catches could be the proximity of baited traps to dogs and cats; the presence of a large gaseous plume (CO_2 exhaled by natural breathing of animals) in the environment may have altered the performance of the CO_2 traps (Carpenter et al., 2008). Because female mosquitoes use multiple cues (*i.e.*, heat, visual, humidity, and odorants) to identify and move toward their hosts (Zhou et al., 2018), the presence of CO_2 in traps might not be sufficient to drive host-seeking mosquitoes toward traps. This is an interesting fact since CO_2 is costly, bulky, short-life and frequently difficult to obtain. The results of this study suggest that its use might not be necessary at sampling sites with high densities of animals. However, these hypotheses should be confirmed by further trials to rule out other unknown factors (*e.g.* traps placement, CO_2 release concentration, etc.) that might be also involved.

Seasonal occurrence showed a high abundance of mosquitoes during early summer and a gradual decline in autumn. On the other hand, adult mosquitoes exhibited a sinusoidal-like dynamic pattern along the sampling period in agreement with other studies from Europe (Bravo-Barriga et al., 2017; Ewing et al., 2019). The trend of adult mosquito catches varied across sampling sites. It showed a first peak upon the emergence from diapause and a second and less pronounced peak at the middle of summer. Mosquitoes readily feed on dogs and cats (Muñoz et al., 2011). In this study, presence of canine blood was demonstrated by sequencing in a blood-engorged *An. maculipennis* s.l. captured at APC1 (data not shown). Several mosquito species, including *An. maculipennis* and *Cx. torrentium*, have been considered as the most likely vectors of filariasis (*D. immitis* and *D. repens*) in canids and felids in Europe (Capelli et al., 2018; Otranto and Dantas-Torres, 2010). *Dirofilaria repens* is the principal agent of human dirofilariosis in the Old World (Capelli et al., 2018). In Spain, both heartworm species have been documented in several geographical areas with variable prevalence in dogs and cats (Cancrini et al., 2000; Capelli et al., 2018). *Dirofilaria immitis* and other unclassified Filaroidea have also been found infecting *Cx. pipiens* s.l. mosquitoes in Spain (Bravo-Barriga et al., 2016). Besides, higher filarial prevalences have been observed among dogs from kennels in comparison to those from veterinary clinics (Cancrini et al., 2000). Thus, the high number of *Cx. pipiens* s.l. in dog and cat kennels observed in this study deserves further attention as potential vectors of heartworm filaroid species in pets.

The high proportion of potential disease vectors, *i.e.*, *Cx. pipiens/Cx. torrentium*, in dog and cat shelters highlights the need for further investigations to assess the prevalence of vector-borne pathogens in flying insects as well as in stray dogs and cats. Active sampling and surveillance programmes in these and other animal shelters would help to keep animals in good health and care conditions as well as to prevent the risk of disease outbreaks. Multisectorial collaboration between entomologists, veterinarians and public health officers might contribute to reduce the impact of vector-borne zoonotic diseases in pets.

Study 3: Mosquitoes in urban areas

IV.3.1. Background

In recent decades, urbanization, and landscape anthropization have caused an impact on the mosquito community composition and abundance worldwide (Wilke et al., 2019). Thus, urbanization has been recognized as a major driver of biodiversity change, often resulting in a decrease in the number of species in urban environments. This loss in mosquito biodiversity in urban areas is a consequence of the simplification of the habitat structures and the alteration of trophic interactions (Ferraguti et al., 2022). However, urbanization increases the availability of man-made water habitats, providing more suitable breeding sites for some native and invasive mosquito species in various types of artificial containers (Ferraguti et al., 2016). Invasive species like *Ae. aegypti* in the US (André B. B. Wilke et al., 2021) or *Ae. albopictus* in Europe have colonized and thrived in urban environments (Roche et al., 2015). As consequence, these changes have led to an increase in the global incidence of MBD, especially in Europe (Medlock et al., 2015).

Urban green areas are the locations in the cities where more vegetation cover can be found. Usually, these areas are destined and designed for the citizens to engage with nature, enjoy leisure time, and perform physical activities (Chiesura, 2004; Medeiros-Sousa et al., 2017). However, these environments harbour the ideal conditions for mosquito proliferation (Medeiros-Sousa et al., 2015, 2017; Zhao et al., 2020). Besides, predators of mosquitoes, such as fish, amphibians, or aquatic invertebrates are usually reduced in these urban environments (Carlson et al., 2004). Additionally, these green ecosystems provide a wide range of animal and human hosts to feed on (André B.B. Wilke et al., 2021). Therefore, these spaces could serve as hotspots for the proliferation of some mosquito species. A characteristic of urban spaces is the high availability of wastewater drainage systems and artificial containers, that when filled with water become habitats for immature stages of mosquitoes (Vezzani, 2007). Also, urban cemeteries are considered suitable habitats for the proliferation of both native and invasive mosquito species (Rydzanicz et al., 2021; Wilke et al., 2020b). These types of environments provide shelter habitats such as bushes and trees for adult mosquitoes as well as immature stage habitats like flowerpots (Vezzani, 2007; Rydzanicz et al., 2021).

Usually, urban areas are generally warmer than periurban and rural areas due to the urban ‘heat island effect’ (Oke, 1973). This effect is caused by the lack of vegetation cover and the presence of cemented areas in these environments (Jenerette et al., 2011). The urban heat island effect shortens the life cycle of mosquitoes, consequently increasing their abundance (LaDeau et al., 2015). Among Culicidae, *Cx. pipiens* s.l. is widely distributed throughout Europe and is the most common species in urban areas (Brugman et al., 2018; Vinogradova, 2000). Besides, species like *Ae. albopictus*, with an important role in different arbovirus transmission, has a higher presence in these human-modified landscapes (Wilke et al., 2021a). This is due to their adaptation and capacity for breeding and developing in artificial and human-made containers (e.g., flowerpots, cans, or buckets), which not all species are able to use (Li et al., 2014). Cemeteries are considered adequate hotspots for these invasive mosquitoes, leading to surveillance efforts in these settings (Abe et al., 2005; Schaffner et al., 2009; Wilke et al., 2020b). Therefore, with cities continuing to expand, it is imperative to monitor the distribution and abundance of mosquito species in green and grey areas. This is crucial for their management and control (Fouet and Kamdem, 2019), and to prevent mosquito-borne diseases.

The aim of the study was to assess the diversity, abundance, seasonal dynamics, larval habitats, and trophic preferences of mosquitoes in urban green areas and urban cemeteries from the three main cities of the Basque Country (northern Spain). A morphological and molecular approach was performed to identify mosquito species, their ecoforms and/or sibling species. In addition, we also evaluated the factors associated with their abundance, species richness, and potential larval sites.

IV.3.2. Material and methods

IV.3.2.1 Study area

The study took place in the three main urban areas of the Basque Country, northern Spain: the *inland city* of Vitoria-Gasteiz (province of Araba), the *coastal city* of Donostia-San Sebastián (province of Gipuzkoa), and the *estuarine city* of Bilbao (province of Bizkaia) (Figure 17). The inland city, Vitoria-Gasteiz, is the capital of the autonomous region of the Basque Country. It has an extension of *ca.* 276 km^2, with around 250,000 inhabitants and a population density of *ca.* 898 inhabitants/km^2 (EUSTAT, 2022, 2021). In 2010, it was awarded by the European Union (EU) the title of 2012 European Green Capital, and in 2019, it was also recognised as a “Global Green City” by the United Nations (UN) due to its environmental policies, green infrastructures, and sustainable mobility (Ayuntamiento de Vitoria-Gasteiz, 2021). The estuarine city, Bilbao, covers an area of 41.60 km^2, with *ca.* 345,000 inhabitants and a population density of *ca.* 8,296 inhabitants/km^2. Until the 1980s, it was primarily an industrial area (Gómez, 1998). The coastal city of Donostia-San Sebastián spans *ca.* 61 km^2, with *ca*.180,000 inhabitants and a population density of around 2,980 inhabitants/km^2 (EUSTAT, 2022, 2021). Both the coastal city and estuarine city enjoy Atlantic climate, characterised by temperate and wet conditions throughout the year. In contrast the inland city has a transitional climate between Atlantic and Mediterranean, with cold winters and drier, warmer summers (Euskalmet, 2022).

Figure 17. Map of the Basque Country indicating the location of the three cities and the six sampling sites: Estuarine city (Bilbao; a. Casilda park; b. Cemetery); Coastal city (Donostia-San Sebastián; c. Kristinaenea park; d. Cemetery); Inland city (Vitoria-Gasteiz; e. Municipal plant nursery; f. Cemetery). [a, c, e: green urban areas (green diamonds); b, d, f: cemeteries (red circles)].

In each city, a green area and a cemetery were selected (Figure 17). The criteria used to choose these sites was based on easy accessibility, suitability, and lower risk of vandalism. The green areas were located in the heart of the selected cities. In Vitoria-Gasteiz, the landscaped area was used mainly for gardening, featuring small tree forest patches, greenhouse area, as well as areas with debris and tyres. In Bilbao, there is an extensive garden with grass, scattered trees, and a large ornamental fountain occupying part of the territory. In Donostia-San Sebastian, there is an extensive, green, leafy, damp and highly vegetated garden with a wide variety of plant and tree species, along with several semi-artificial water pools. A list of the hosts that were most frequently observed during field visits is provided (Table 11).

Table 11. Commonly encountered potential hosts identified during field visits to green areas.

City	Green areas
Vitoria-Gasteiz	European rabbit (*Oryctolagus cuniculus*) Gardeners and walkers (*Hommo sapiens*) Diverse forest Passeriformes (*Erithacus rubecula, Parus major, Cyanistes caeruleus, Turdus merula, Pica pica*) Eurasian hoopoe *(Upupa epops)* and common wood pigeon *(Columba palumbus)*
Bilbao	Swan (*Cygnus* sp.) Peafowl (*Pavus cristatus*) Gardeners and walkers (*Hommo sapiens*) Diverse Anatidae Common pigeon (*Columba livia*)
Donostia-San Sebastián	Swan (*Cygnus* sp.) Peafowl (*Pavus cristatus*) Gardeners and walkers (*Hommo sapiens*) Diverse Anatidae Diverse forest Passeriformes: *Turdus philomelos* and *Erithacus rubecula* Common pigeon (*Columba livia*)

The three selected cemeteries were different in terms of structure, vegetation, and mainly on the number of water-holding containers, probably reflecting distinctive cultural habits. All cemeteries were soil-cemented and had mixed trees and green patches.

IV.3.2.2 Field sampling approach

Mosquito trapping was carried out fortnightly during two periods: from 1-May to 31-October 2019 and from 1-June to 30-November 2020. COVID-19 pandemic in 2020 forced to start the field sampling one month later. Two CDC-miniature traps (model 1212, John Hock, USA) were deployed in each sampling site, equipped with incandescent light, and baited with *ca.* 1.5 kg of dry ice (CO_2). Traps were placed at least 100 meters apart and were placed on two distinctive habitats. They were positioned in shady, humid, and windless areas as these are locations where mosquitoes tend to rest. All traps were set up early in the morning and recovered 24 h later. In addition, in 2019, mosquito larval sites were searched and sampled once per month in a radius of 200 m around CDC traps. In green areas, immature mosquito stages were collected using a dipper (600 ml) as detailed by González et al. (2021). These sampled were then transported to the laboratory and kept in mosquito Breeders (Bioquip Products, U.S.A.) until adult emergence. A total of 7, 4 and 5 types of larval sites were inspected each month in the inland city, estuarine city, and coastal city, respectively. In cemeteries, instead of sampling around a 200 m radius of each CDC trap position, we sampled a maximum surface of 2,500 m^2 per site. The cemeteries of the inland and estuarine cities contained a low number of containers (n = 149), whereas the cemetery in the coastal city included more than 340 containers. The number of containers inspected, number of containers with water, and number of containers positive to mosquitoes are detailed in Table 12.

Table 12. Number of containers inspected in each cemetery, with water and positive (presence of larvae), by city and month.

Parameters	City	Month					
		May	June	July	August	September	October
nº of containers inspected	Vitoria-Gasteiz	16	12	9	7	6	5
	Bilbao	25	25	20	9	9	6
	Donostia	62	42	58	41	26	112
nº of containers with water	Vitoria-Gasteiz	9	7	0	3	3	3
	Bilbao	15	14	6	5	7	2
	Donostia	38	29	26	37	21	78
nº of containers positive	Vitoria-Gasteiz	0	0	0	0	0	0
	Bilbao	0	0	0	1	2	0
	Donostia	0	4	6	5	5	7

IV.3.2.3 Mosquito species identification

In the laboratory, mosquitoes were sorted by sex and physiological status (blood-fed, gravid, and unfed). The species identification relied on morphological features of females and male genitalia using taxonomic keys (Becker et al., 2020; Schaffner et al., 2001). Examination of genitalia of males allowed for differentiation between *Cx. torrentium* and *Cx. pipiens* s.l. Damaged or morphologically indistinguishable mosquito specimens were identified by molecular methods. Briefly, genomic DNA extraction was carried out with NZY Tissue gDNA isolation kit (NZYTech, Lisboa, Portugal) followed by a PCR targeting the Cytochrome c Oxidase I (COI) with primers C1-J-1718 and C1-N-2191 as described by Delgado-Serra et al. (2021). PCR amplicons were then purified using ExoSAP-IT (Applied Biosystems, Thermo Fisher Scientific, Vilnius, Lithuania) and submitted for Sanger sequencing (Eurofins Genomics, Germany). The sequences obtained were analysed using Geneious Prime software (v.2022.2.2) and compared with the GenBank database through nucleotide sequence homology searches on the network server of the National Center for Biotechnology Information (NCBI) using BLAST or at the BOLD Database (http://www.boldsystems.org/index.php).

Mosquitoes belonging to the *Anopheles maculipennis* s.l. complex were identified to the species level using a PCR-RFLP assay targeting polymorphisms in the ITS-2 (Vicente et al., 2011) with primers described in Collins and Paskewitz (1996). Similarly, a subsample of the specimens morphologically identified as *Cx. pipiens* s.l. of each green area and cemetery were analysed by molecular methods (ca. 25%; n = 146; 44 from 2019 and 102 in 2020) to determine their ecoform (*Cx. pipiens pipiens*, *Cx. pipiens molestus* and its hybrids) by targeting the flanking region of the CQ11 microsatellite (Bahnck and Fonseca, 2006).

IV.3.2.4 Host blood-meal analysis

Vertebrate host species of blood-fed and gravid females collected in 2019 were investigated at the Centre for Biodiversity Genomics, University of Guelph (Guelph, ON, Canada). Host feeding patterns were identified using a metabarcoding-like approach with NGS technology as previously described (Estrada-Franco et al., 2020; González et al., 2022, 2020). Identification was considered valid only when the query sequence matched the reference sequence with at least 95% nucleotide identity. Detailed specimen records and sequence information were uploaded to the BOLD Database (http://www.boldsystems.org) and can be found within the Working Group 1.4 Initiative “Human Pathogens and Zoonoses” container “MCBCS-Surveillance of mosquitoes and *Culicoides* in the Basque Country, Spain.” The digital object identifier (DOI) for publicly available projects in BOLD is doi: dx.doi.org/10.5883/DS-MQBMBC.

IV.3.2.5 Data analysis

Statistical analyses were performed using R statistical software version 4.2.0 (R Core Team, 2022). Differences between the abundance of the most trapped mosquito species in green areas and cemeteries were analysed using non-parametric Wilcoxon Rank Sum Test. Chi-squared test and Fisher exact test were used to evaluate the differences between *Cx. pipiens* ecoforms and the type of sampling area (green area *vs.* cemetery) and city. Multivariate Generalized Linear Models (GLM) were run to evaluate the differences in the overall abundance of mosquitoes (catches/trap/night), related to sampling site (green urban area, cemetery), city (Vitoria-Gasteiz, Bilbao, Donostia), year of sampling (2019, 2020) and month of sampling (June to October, shared period for both periods of sampling). A Negative Binomial generalized linear model (NBGLMs) was employed (O´Hara and Kotze, 2010) due to the data over-dispersion of the mosquito abundance, using the MASS package (Venables and Ripley, 2002). Using the “MuMIn” package and “dredge” function (Barton, 2020), the best models were selected based on AIC and corrected to sample size (AICc). The overall fit of the model was evaluated with a likelihood ratio test, comparing the best model with the null model. Species richness - S, and Shannon Wiener’s diversity - H’, were calculated to compare biodiversity among cities, sampling areas, and type of sampling sites (green area *vs.* cemetery) using the “diversity” function in the “vegan” package (Oksanen et al., 2022).

IV.3.3. Results

IV.3.3.1 Species composition and abundance

A total of 846 mosquitoes (682 females and 164 males) were collected by CDC suction traps in green areas and cemeteries from the Basque Country (northern Spain). In 2019, a fewer number of mosquitoes (n = 263, 207 females and 56 males) were captured when compared with the catches in 2020 (n = 583 mosquitoes, 475 females and 108 males).

Morphological and molecular analyses allowed to the identification of 21 mosquito species (one invasive and 20 native mosquitoes), encompassing six *Aedes* spp., seven *Culex* spp., four *Anopheles* spp., three *Culiseta* spp., and one *Coquillettidia* species (Table 13). Among them, three species (*Cx. pipiens* s.l., *Cs. longiareolata*, and *Cx. hortensis*) were found in the three cities. The three specimens initially included as *An. maculipennis* s.l. complex were subsequently identified as *An. atroparvus* (n = 1) and *An. maculipennis* s.s. (n = 2). Vitoria-Gasteiz - harboured the highest species richness with 13 different species, followed by Donostia (n = 11), and Bilbao (n = 8) (Figure 18). Regarding abundance, the highest mean abundance belonged to the green area of Bilbao (6.16 ± 1.37) and the lowest mean the cemetery in Vitoria-Gasteiz (1.20 ± 0.26) (Table 13). Overall mean abundance of green areas was higher (4.13 ± 0.55) than cemeteries (1.51 ± 0.17) (W = 8244; $p < 0.001$). Interestingly, a single specimen of *Ae. albopictus* was captured with CDC traps in the cemetery of Bilbao (in 2020). *Culex pipiens* s.l./*Cx. torrentium* (n = 622, 73.5%) was significantly the most abundant species (W = 51945; $p < 0.001$), followed by *Cs. longiareolata* (n = 118, 13.9%). In all sampled areas, the most captured species was *Cx. pipiens* s.l./*Cx. torrentium*, being more abundant in green areas (76.7% of the total catches, 476/621) than in cemeteries (64.9% of the total catches, 146/225) (W = 8644, $p < 0.001$).

The molecular analysis yielded 65 barcoding COI sequences of 443–525 bp in length with 97–100% homology when compared with GenBank sequences. A selection of these sequences (n = 21 and 13 species) was deposited in GenBank under accession numbers PP218317-PP218337.

Table 13. Culicidae trapped by baited CDC traps in the six urban environments in the Basque Country in 2019 and 2020.

Culicidae	**Green areas**			**Cemeteries**			**Total**
	Bilbao	**Donostia**	**Vitoria-Gasteiz**	**Bilbao**	**Donostia**	**Vitoria-Gasteiz**	
Ae. albopictus	0	0	0	1	0	0	1
Ae. caspius	1	0	0	0	0	0	1
Ae. detritus	0	1	0	0	0	0	1
Ae. geniculatus	0	9	1	0	0	0	10
Ae. rusticus	0	0	6	0	0	0	6
Ae. vexans	0	1	4	0	0	0	5
An. atroparvus s.s.	0	0	1	0	0	0	1
An. claviger	0	0	12	0	0	1	13
An. maculipennis s.s.	0	0	2	0	0	0	2
An. plumbeus	0	8	0	0	3	0	11
Cs. annulata	0	13	7	0	2	1	23
Cs. longiareolata	37	12	12	5	44	8	118
Cs. subochrea	0	0	1	0	0	0	1
Cx. hortensis	0	0	0	3	6	1	10
Cx. mimeticus	0	1	0	0	0	0	1
Cx. modestus	1	0	0	0	0	0	1
Cx. pipiens s,l./ *Cx. torrentium* [a]	267	144	65	51	48	47	622
Cx. territans	0	0	1	0	0	0	1
Cx. theileri	0	0	6	0	0	1	7
Cq. buxtoni	0	5	0	1	0	0	6
Unidentified species	2	0	0	0	2	1	5
Total	**308**	**194**	**118**	**61**	**105**	**60**	**846**
MEAN ± SE	6.16±1.37	3.88±0.53	2.36±0.66	1.22±0.29	2.10±0.33	1.20±0.26	3.32±0.39

[a] At least, five specimens of *Cx. torrentium* were identified by morphological (1 male in the cemetery of Donostia) or molecular methods (3 females in the green area of Donostia and 1 female in the cemetery of Bilbao). Males and females were pooled.
SE: Standard Error

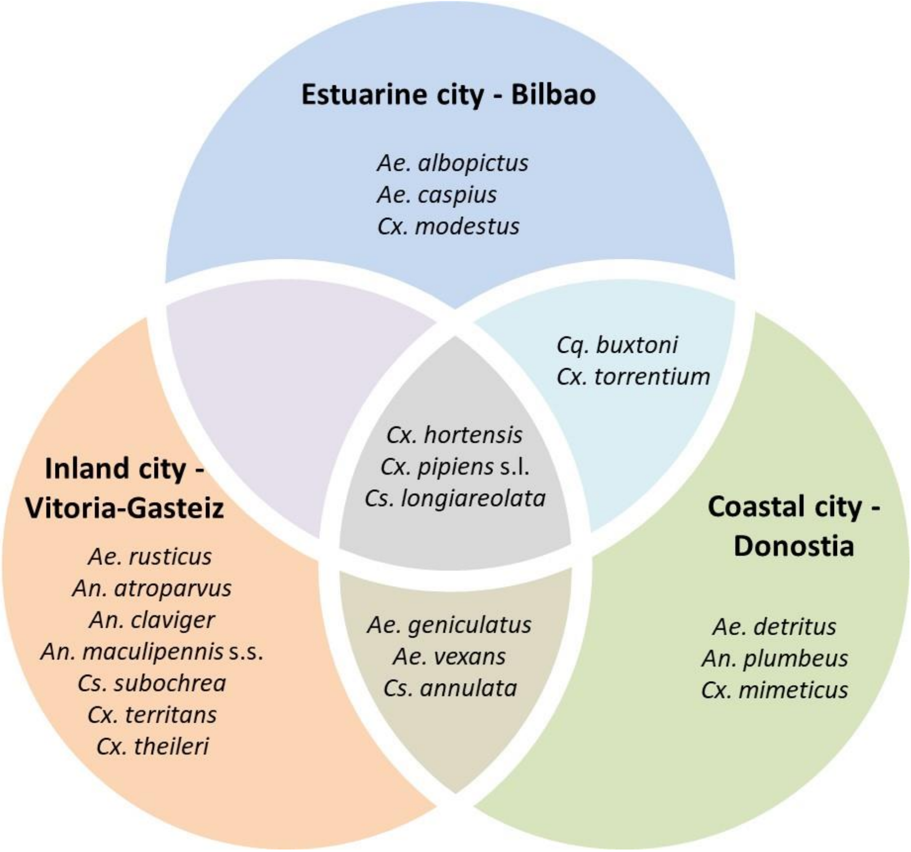

Figure 18. Venn diagram represents the Culicidae species found in urban environments of the three main cities of the Basque Country.

Shannon's Diversity Index showed a higher diversity in Vitoria-Gasteiz (H' = 1.39), followed by Donostia (H' = 1.24), and Bilbao (H' = 0.49). The diversity was identical in both green areas (H' = 1.03) and cemeteries (H' = 1.03). The highest diversity index was found in the green area of Vitoria-Gasteiz (H' = 1.61) (Figure 19).

The approach to determine *Cx. pipiens* ecoforms showed *Cx. pipiens pipiens* (n = 82, 56.2%) as the most abundant, followed by the *Cx. pipiens molestus* (n = 46, 31.5%), and the hybrid form (n = 18, 12.3%) (Figure 20). Overall, no significant differences were found between green area and cemetery in the distribution of the three ecoforms (χ^2 = 1.185, p = 0.553). No significant differences were found among the three cities (χ^2 = 4.366, p = 0.359) being the ecoform *pipiens* the most abundant in all the cities, followed by the form *molestus* (Figure 20).

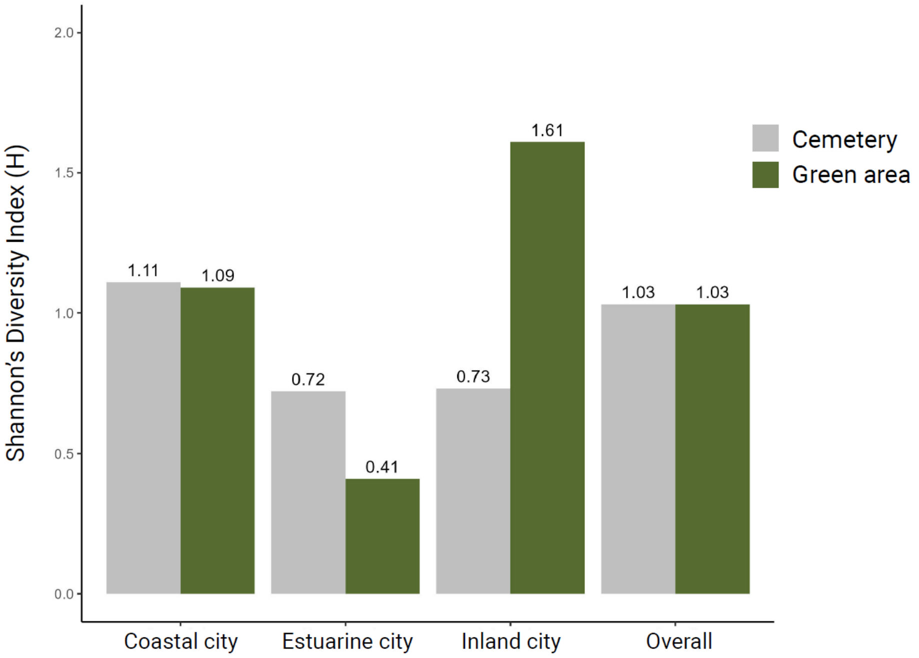

Figure 19. Shannon's Diversity Index (H) by city and type of sampling site. [coastal city - *Donostia-San Sebastián;* estuarine city – *Bilbao;* inland city - *Vitoria-Gasteiz*]

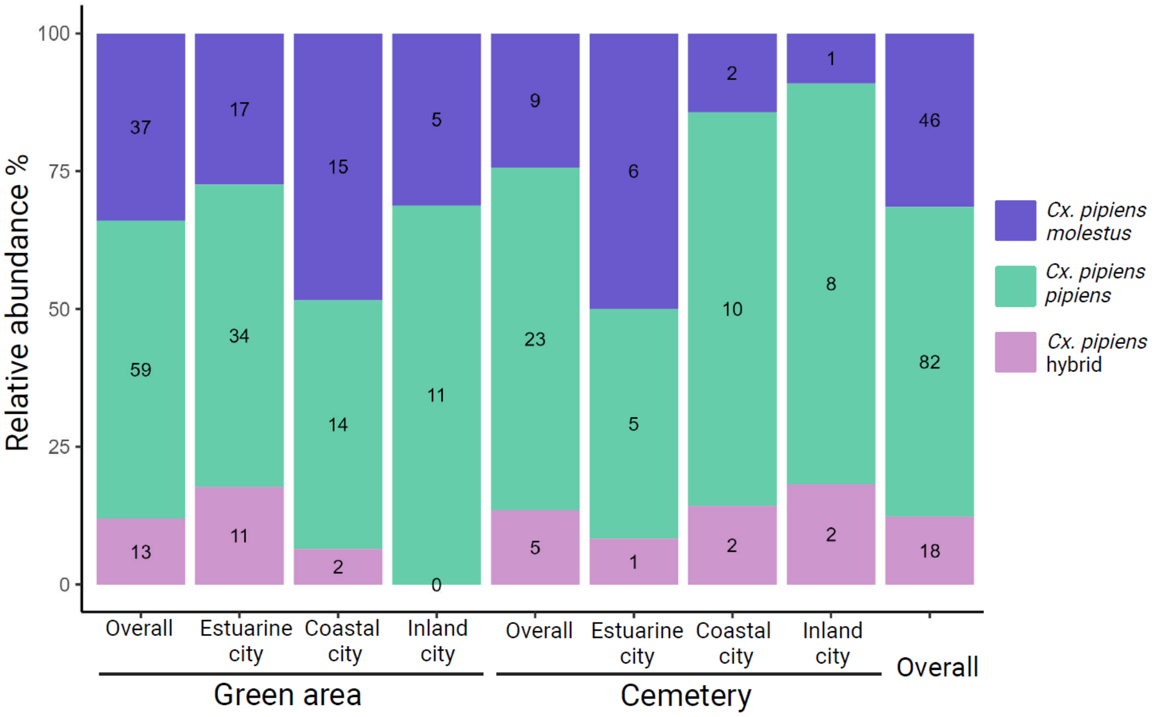

Figure 20. Relative abundance of *Cx. pipiens* s.l. ecoforms in the six urban environments in the Basque Country [number inside the bars correspond to absolute numbers] [estuarine city – *Bilbao;* coastal city - *Donostia-San Sebastián;* inland city - *Vitoria-Gasteiz*].

IV.3.3.2 Seasonal activity

Considering the mosquito catches obtained by CDC traps in 2019 and 2020, mosquito flight activity extended throughout the entire sampling period. Population dynamics during 2019 in the three cities showed a moderate increase during the first months of sampling, with peaks in June and August in the coastal city (Donostia), in July and August in the estuarine city (Bilbao), and June, August and October in the inland (Vitoria Gasteiz) (Figure 21). In 2020, Bilbao showed the highest mosquito abundance. All the cities experienced an increase in mosquito abundance during the initial months of sampling, followed by a progressive decline from August onwards (Figure 21).

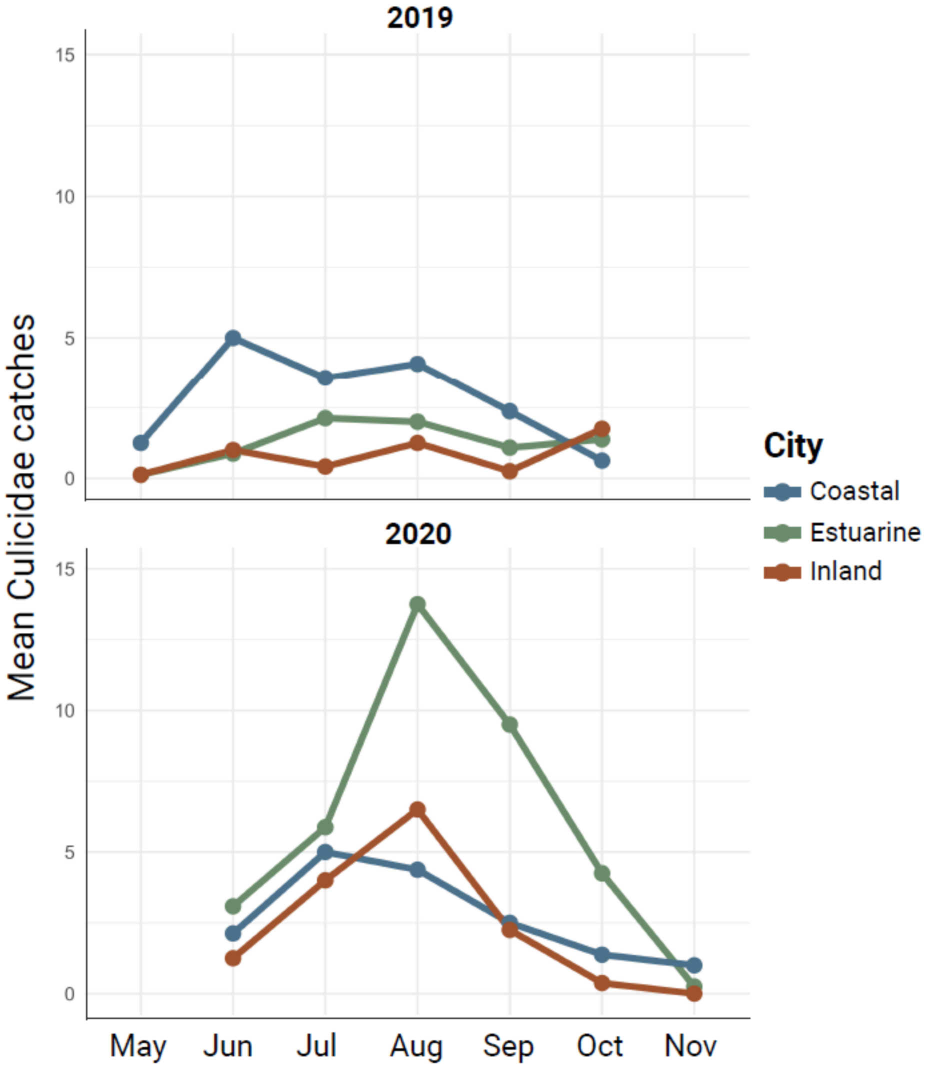

Figure 21. Seasonal flight activity of Culicidae captured in CDC traps at the three cities of the Basque Country in 2019 and 2020 [coastal city - *Donostia-San Sebastián;* estuarine city – *Bilbao;* inland city - *Vitoria-Gasteiz*].

IV.3.3.2 Larval rearing sites

Eight different mosquito species were identified, including *Cx. pipiens*, *Cx. torrentium*, *Cx. hortensis*, *Cx. territans*, *An. claviger*, *Cs. annulata*, *Cs. longiareolata*, and *Ae. geniculatus*. Most larvae were collected from green areas (n = 319) when compared with cemeteries (n = 143) (Table 14). Overall, *Cx. pipiens* s.l. (n = 340, 73.6%) was the most abundant species in the larval sites from all the sampling areas, followed by *Cs. longiareolata* (n = 69, 15.0%) (Table 14). The most prolific mosquito larval sites in green areas were pools of water (semi-artificial ponds), diverse water-holding containers (tyres and buckets), and drainage systems, while in cemeteries flowerpots supported most of the larval abundance and pots and funeral urns into a lesser extent. Larvae were found in all type of water-holding materials in cemeteries (plastic, ceramic, metal and/or marble). *Culex pipiens* s.l. and *Culiseta* spp. bred in a wide variety of artificial and natural water-holdings whereas *Ae. geniculatus* preferred to rear exclusively on tree-holes together with *Cx. pipiens*, *Cx. territans,* and *Cx. torrentium*, into lesser extent. The latter was found cohabiting in the same habitats as *Cx. pipiens*. Interestingly, a single *An. claviger* was found breeding in a large plastic tray. It is interesting to note that artificial urban lakes might contain larvae of *Cx. pipiens* on the muddy and shady edges. Huge differences were recorded in the mosquito abundance among the three study sites which was a reflect of the number of available larval sites, i.e., in the green area of the estuarine city and its cemetery a scarce number of developmental sites were found.

Table 14. Culicidae species found in larval habitats in the six urban environments studied in the Basque Country during 2019.

Culicidae species	**Green areas**				**Cemeteries**				**Total**
	Bilbao	**Donostia**	**Vitoria-Gasteiz**	**Total**	**Bilbao**	**Donostia**	**Vitoria-Gasteiz**	**Total**	
Cs. annulata		7	1	8					8
Cs. longiareolata		3	28	31		38		38	69
Cx. pipiens s.l./ *Cx. torrentium*	21	114	118	253	30	57		87	340
Cx. hortensis						18		18	18
Cx. territans		19		19					19
An. claviger			1	1					1
Ae. geniculatus		7		7					7
Total	**21**	**150**	**148**	**319**	**30**	**113**	**0**	**143**	**462**

IV.3.3.3 Analyses of variables affecting mosquito abundance

The negative binomial model showed a positive association between the total abundance of mosquitoes with the type of sampling area, being significantly higher in the green areas compared to the cemeteries (Table 15). Moreover, the abundance of mosquitoes collected in CDC traps was also positively associated with the city, the year, and the month of sampling, being the abundance significantly higher in Donostia and Bilbao, in the year 2020, and in the months of July and August (Table 15).

Table 15. Summary of best negative binomial regression model for total number of Culicidae per CDC trap and night.

Variables	Abundance per CDC trap/night		
	Est ± SE [1]	**z** [2]	**p-value** [3]
Type of sampling area			
Cemetery	Ref. [4]		
Green Area	0.90 ± 0.16	5.66	< 0.001
City			
Inland – Vitoria-Gasteiz	Ref.		
Coastal - Donostia	0.66 ± 0.20	3.36	< 0.001
Estuarine - Bilbao	0.59 ± 0.20	2.94	0.003
Year of sampling			
2019	Ref.		
2020	0.74± 0.16	4.64	< 0.001
Month of sampling			
October	Ref.		
June	0.32± 0.26	1.20	0.229
July	0.63 ± 0.25	2.49	0.013
August	0.98 ± 0.25	3.84	< 0.001
September	0.31 ± 0.27	1.21	0.227

[1] Est ± SE = Estimate ± Standard Error; [2] z = statistic z-value; [3] p = p-value; [4] Ref. = reference category

IV.3.3.4. Host DNA blood-meals

Few blood-fed/gravid specimens were captured. Amplification success in mosquito samples was 47.1% (8/17). Female mosquitoes that failed in identifying DNA host were categorised with advanced Sella stages. *Culex pipiens* s.l. fed on seven species of urban birds (Table 16). It was not possible to identify the host-DNA blood meal in five *Culiseta* spp.

Table 16. Blood meal host identification in Culicidae in the six urban environments studied in the Basque Country during 2019.

	Culicidae		
City	**No.**[1]	**Species**	**Host DNA (No.)** [2]
Estuarine city - Bilbao	3	*Cx. pipiens* s.l.	*Turdus merula* (1)
Coastal city - Donostia	1	*Cs. annulata*	-
	4	*Cs. longiareolata*	-
	9	*Cx. pipiens* s.l.	*Turdus merula* (1) *Turdus philomelos* (1) *Anas platyrhynchos* (1) *Erithacus rubecula* (1) *Passer domesticus* (1) *Serinus serinus* (1) *Serinus canarius* (1)

[1] Total number of blood-fed or gravid specimens analysed. [2] In parentheses: the number of mosquitoes in which host DNA was identified.

IV.3.4. Discussion

Globalization and landscape anthropization affect the mosquito community composition, distribution, and abundance in urban areas, consequently influencing the incidence of mosquito-borne diseases (Manguin and Boete, 2011; Wilke et al., 2021a). Mosquito abundance is linked to landscape composition in urban green spaces (Ferraguti et al., 2016; Kache et al., 2022; Zhao et al., 2020). For example, in some locations an increased presence of water and aquatic plants is correlated with a higher mosquito density, while greater coverage of woodland plants appears to reduce mosquito abundance (Zhao et al., 2020). However, other studies show that the distribution of mosquitoes depends on the life stage and the influence of microclimatic conditions (Krol et al., 2024). For this reason, we aimed to widen the study of Culicidae in northern Spain, to determine which species inhabit urban areas and assess their potential risk to public health. The number of mosquito species and their abundance in the current study was slightly lower compared to pristine habitats of the Study I. Overall, mean abundance per trap and night was low but similar to findings reported in urban areas from other Spanish regions (Bravo-Barriga et al., 2017; Ferraguti et al., 2016), with *Cx. pipiens* being the most abundant species. *Culex pipiens* is the most common and predominant species in urban areas both in Spain and in the northern hemisphere and serves as a vector of several diseases such as WNV (Gangoso et al., 2020). In this study, this species was found breeding in practically all kind of habitats (15/16 types), regardless their natural or artificial nature. This species has two ecologically distinct ecoforms and a hybrid; the *molestus* form is usually described as the belowground form, mammophilic, and capable of laying eggs without a bloodmeal (Becker et al., 2020), while the *pipiens* form is an ornithophilic aboveground form (Haba and McBride, 2022). This ecoform categorization is well-defined in northern parts of Europe, but in southern Europe, this distinction is not as clear (Haba and McBride, 2022). In our study, *Cx. pipiens pipiens* was the most abundant form followed by *Cx. pipiens molestus*, in accordance with other studies carried out in urban areas of the Iberian Peninsula (Bravo-Barriga et al., 2017; Osório et al., 2014). Both ecoforms exhibit a pronounced preference for urbanized areas (Bravo-Barriga et al., 2017; Osório et al., 2014), despite in other Spanish regions *Cx. pipiens pipiens* are more frequently found in natural areas (Martínez de la Puente et al., 2016). Interestingly, this study showed no differences in the frequency of these forms between green areas and cemeteries. Regarding hybrids, the percentage found in this study (12.3%) is lower compared to the observed in other Spanish regions (Bravo-Barriga et al., 2017). These differences might be attributed to host availability and habitat conditions, which might favour hybridization.

Along with *Cx. pipiens* s.l., *Cs. longiareolata* is considered the second most abundant and widely distributed species in many regions of Spain (Bueno-Marí et al., 2012). This mosquito species is commonly found breeding in artificial containers in urban environments (Roiz et al., 2007). In our study, this species was recorded developing in artificial habitats (track tyres, buckets, flowerpots, funeral urns, small pools of water, and sewer systems) in both green areas and cemeteries. *Culiseta longiareolata* is considered ornithophilic (Becker et al., 2020) but also feeds on mammals, including humans (Osório et al., 2012). Although it has been considered of low interest for public health, this species could potentially act as vector of avian pathogens (Schaffner et al., 2001).

Our study also showed that the highest mosquito abundance was recorded in the green urban areas, especially in 2020, when COVID-19 pandemic took place. This difference could be explained by climatic variables (not registered in this study) or by the management of the green areas, which due to the pandemia, were neglected and presumably more resting sites and hides were available when compared in the previous year. The green areas investigated in this study have shown a higher diversity of mosquitoes than the cemeteries. Most of the species found in green areas and cemeteries have been reported in previous studies conducted in the territory. *Aedes geniculatus*, however, has only been previously identified through egg analysis by molecular tools (see Study IV). This mosquito species exhibited aggressive biting behaviour towards humans during the field work. Small size water-filled holes in alder trees (*Alnus* sp.) were used for this species as developmental sites in line with other studies (Müller et al., 2012). It is interesting to note that most of the species found in our study have been also recorded in nearby naturalised areas (Study I). This is the case of *Ae. caspius*, *Ae. detritus,* and *Cx. modestus* which usually breed in brackish and saline water (Hawkes et al., 2020; Medlock and Vaux, 2015a;

Roiz et al., 2015), have been found in estuarine and coastal cities, respectively. *Aedes rusticus* is abundant in naturalized areas located near the inland city (Study I) in the transition zone between Atlantic and Continental Mediterranean climates. At present, this species has not been found in the Atlantic climate area. In our study, only five specimens of *Ae. rusticus* were found in the green area from the inland city which suffers more extreme climatic conditions than the estuarine and coastal cities, located both near the coast and influenced by the mild Atlantic climate. This could explain the limits of the distribution of *Ae. rusticus*. From other species found in green areas, such as *Cx. mimeticus*, *Cs. subochrea*, *An. atroparvus* or *An. maculipennis* s.s., only one or two specimens were collected, making difficult to reach any conclusion about distribution and habitat preferences.

Cemeteries are widely recognized as areas of high mosquito productivity and are considered ideal locations for the proliferation of mosquitoes as they provide multiple habitats for the mosquitoes to develop (Schaffner et al., 2009). Urban cemeteries are also very accessible and are frequently visited which provides blood sources either from visitors or on-site workers (Koban et al., 2019). However, the productivity of cemeteries as reservoir of immature larvae of mosquitoes depend on many factors, such cultural practices and religious customs (Vezzani, 2007). This explained that the cemetery in Donostia was very productive in terms of number of water-filled containers, particularly flowerpots and funeral urns, and in contrast, larval sites in the other two places were scarce or inexistent. It may be due to the management of the individual cemeteries, where visitors are encouraged to minimize the flowerpots in the graves and monuments. Cemeteries are also routinely surveyed in Europe for the monitoring *Aedes* invasive species such as *Ae. albopictus*, *Ae. japonicus* and *Ae*. *koreicus* (Hohmeister et al., 2021; Kampen et al., 2016a; Koban et al., 2019). In fact, in our study a single specimen of *Ae. albopictus* was captured in one of the three cemeteries investigated, despite this invasive species is already present in the Basque Country since 2014 (Goiri et al., 2020). Although the tiger mosquito is currently widely distributed in the region (see Study IV), it seems that the population density at the time of sampling was still not high enough to be collected. It is also interesting to note that a second *Aedes* invasive species (i.e. *Aedes japonicus*) present in the Basque Country (Eritja et al., 2021; González et al., 2022) was not recorded in this study, most likely attributed to many reasons, such as the vegetation and woodland were not abundant in the cemeteries sampled.

Regarding developmental sites of immature larvae of mosquitoes, our study showed that multiple artificial containers in cemeteries might serve as larval sites for mosquito species such as *Cx. pipiens* s.l./ *Cx. torrentium*, *Cs. longiareolata* and *Cx. hortensis*. Interestingly, *Cx. hortensis* is the mosquito species that has been detected only in cemeteries, captured by both, CDC traps and dipping. Little is known about the phenology and general biology of *Cx*. *hortensis* (Becker et al., 2020), but this species is frequently found in Spain, France, Italy, and Greece up to central Europe (Robert et al., 2019). In our study, *Cx. pipiens* s.l. was the most abundant species sampled in the immature stage but also the most abundant species captured as an adult. However, in previous studies carried out in the same area, *Culex hortensis* was the most abundant species found in larval habitats in urban areas (González et al., 2021).

From the 21 species of mosquitoes identified in cemeteries and urban green areas, *Ae. albopictus* has a real impact on human health. It is a competent vector of several arboviruses and has been implicated in the European autochthonous transmission of these diseases in recent years (Giunti et al., 2023a). *Culex pipiens* s.l*., Cx. modestus*, and *Ae. detritus* have been confirmed also to be vectors of WNV in Europe (Mancini et al., 2017; Soto and Delang, 2023; Vilibic-Cavlek et al., 2019), with *Cx. pipiens* s.l. being one of the most important vector of this virus (Soto et al., 2023; Vogels et al., 2017a). Other mosquito species such as the ornithophilic *Ae. vexans*, which primarily feed on birds*,* have been found to be a competent vector in the transmission of WNV, acting as a bridge vector between birds and humans (Anderson et al., 2020; Tiawsirisup et al., 2008). *Anopheles plumbeus* has gained interest in Europe as it has been identified as a potential malaria vector (Bertola et al., 2022; Bueno-Marí and Jiménez-Peydró, 2011a; Schaffner et al., 2012). Besides, among the members of the *An. maculipennis* complex, it is relevant to note the detection of *An. atroparvus* in the green area of the inland city, as it is a recognised historic vector of malaria (Bertola et al., 2022).

Blood-meal analysis is a fundamental tool to understand the ecology of mosquitoes (Yan et al., 2021), as feeding habits of mosquitoes are critical factors in the transmission of vector-borne pathogens (Montgomery et al., 2011). Owing to the low number of blood-fed specimens recorded in our study, it is difficult to establish robust conclusions. Nonetheless, the trophic habits of *Cx. pipiens* s.l. showed strong ornithophilic preferences. They took blood meals from the local avifauna observed in the urban areas of the study area in line with previous works performed also in urbanised habitats (González et al., 2020), even when other mammal hosts were available nearby, such as walkers and dogs. However, in the metropolitan area of Barcelona, *Cx. pipiens* showed preference for birds but also for humans, dogs, and cats (Muñoz et al., 2011). Host choice is host dependent as showed in the urban zoos of Barcelona where *Cx. pipiens* also showed mixed feeding habits (Martínez de la Puente et al., 2020).

This study provides new insights on the abundance and mosquito community composition in green urban areas and cemeteries of the Basque Country. It highlights the differences among cities with varying urban planning and population density. Despite the relatively low abundance of mosquitoes in urbanized areas, the most commonly trapped species are regarded as crucial vectors for various pathogens. Therefore, health authorities should adopt a multi-faceted approach to mosquito management, including the implementation of biological treatments in mosquito breeding sites and the removal of water-filled containers. Other effective strategies may include community engagement and education programs to raise awareness about reducing stagnant water areas in residential and public spaces. Regular inspection and maintenance of drainage systems to prevent water accumulation, as well as the use of environmentally friendly larvicides to target mosquito breeding grounds, could further bolster control measures. Collaborative efforts between health departments, local authorities, and community participation can enhance the efficacy of control programs aimed at reducing mosquito populations and the risk of associated disease transmission. Besides, the current results indicate that *Cx. pipiens* s.l. is the most common taxon of the Culicidae family in urban areas, exhibiting an ornithophilic feeding preference. A better understanding of the trophic behaviour/preferences of these Diptera pests can contribute to understand the transmission patterns of pathogens of public health interest.

Study 4: Invasive *Aedes* spp. mosquitoes in urban and peri-urban areas of the Basque Country

IV.4.1. Background

Globalization, human travel, and global trade have facilitated the spread of exotic invasive mosquitoes of the genus *Aedes* (Medlock et al., 2012b). Additionally, climatic and environmental changes increase the possibility of the spread and establishment of some species in new regions. In Europe, six species of *Aedes* invasive mosquitoes (AIM) (*Ae. albopictus*, *Ae. aegypti*, *Ae. japonicus*, *Ae. koreicus, Ae. atropalpus,* and *Ae. triseriatus*) have been introduced since the 1970s (Miranda et al., 2022) and some have known established populations (ECDC, 2023c; Medlock et al., 2015). Among them, only two species are present in Spain (Miranda et al., 2022).

Aedes albopictus (Skuse, 1894) is an exotic invasive species that was introduced into Albania during the late 1970s and later in Italy in 1990 (Adhami and Reiter, 1998; Dalla Pozza and Majori, 1992) and is currently widely distributed in Europe. Its ability to adapt to cold temperatures and overwinter in temperate regions and its plasticity to adapt to different habitats, together with globalization and climate change, contribute to the successful invasion of this multivoltine species (Giunti et al., 2023a). Urban emplacements provide suitable habitats and resources for *Ae. albopictus* development, allowing them to colonize and proliferate; It is considered a container-breeding mosquito, and its ability to occupy habitats in urban environments is noticeable. This species represents a public health concern since it can act as a vector of some arboviruses, most notably dengue, chikungunya and Zika (Giunti et al., 2023a). In fact, several autochthonous outbreaks have been recently reported in Europe, thus confirming local transmission of these diseases in places where *Ae. albopictus* has been established (Cochet et al., 2022; Delisle et al., 2015; Lazzarini et al., 2020; Monge et al., 2020; Riccardo et al., 2019). The epidemiology of vector-borne pathogen transmission is driven by the interaction between vector, host, and pathogen, and is profoundly affected by urbanization processes. As urbanization processes increase globally, many countries are experiencing the re-emergence and introduction of vector-borne diseases (Wilke et al., 2021a). In addition, the aggressive biting behavior of *Ae. albopictus* jeopardizes the quality of life of local citizens and can even have a direct impact on the regional economy. In Spain, after the first detection of *Ae. albopictus* in Catalonia in 2004, the Spanish Ministry of Health launched a surveillance campaign in 2007 in several Mediterranean Spanish regions (Collantes et al., 2015). In northern Spain, *Ae. albopictus* was identified for the first time in 2014 in the Basque Country, on the border with France, and since then, it has been expanding toward new areas (Goiri et al., 2020).

Aedes japonicus (Theobald, 1901) was first detected in 2000 in France in a storage yard of imported tires (Schaffner et al., 2003). Since then, this species has been reported mostly in central Europe (Kampen and Werner, 2014; Koban et al., 2019). *Aedes japonicus* is well-adapted to temperate climates and is capable of withstanding cold and snowy winters. It is a multivoltine species, and larval hatching occurs early in the year as soon as breeding sites lose their ice cover at water temperatures of 4.0-4.5 °C (Kampen and Werner, 2014). This species has an adaptive capacity, and they have been observed to displace other indigenous mosquito species (Kaufman and Fonseca, 2014). The activity period of adult mosquitoes can last until early December. In contrast to *Ae. albopictus*, this species prefers forested and bushy areas, and larvae can be found in tree holes, tree stumps, rainwater pools (Kampen and Werner, 2014), and other natural or artificial containers. *Aedes japonicus* is a potential vector of several viruses of medical and veterinary importance, such as chikungunya, dengue, Zika, and West Nile (Martinet et al., 2019). This species is not considered a primary nuisance species to humans (Cebrián-Camisón et al., 2020), but it is of particular concern because of its ability to rapidly adapt to new habitats given its high tolerance to a broad range of climate conditions (Kaufman and Fonseca, 2014; Montarsi et al., 2019). *Aedes japonicus* was detected in northern Spain (Asturias) in 2018 (Eritja et al., 2019) and two years later was identified in two close regions (Cantabria and the Basque Country) (Eritja et al., 2021; Miranda et al., 2022), suggesting a new colonization area in Europe.

Urbanization processes have a major impact on mosquito communities by decreasing species richness and increasing the abundance of selected mosquito species such as *Ae. albopictus*, which are very well-adapted to urban and suburban ecosystems (Bartlett-Healy et al., 2012; Wilke, et al., 2021a). Until now, during the surveillance program carried out in the Basque Country, the selection of sampling areas was focused on places with heavy road traffic (such as car parks, petrol stations, or logistics platforms, among others) to increase the probability of detecting *Ae. albopictus* (Goiri et al., 2020). However, recent findings (Eritja et al., 2021; Miranda et al., 2022) lead us to think that, *Ae. japonicus* could also be established in this area. Thus, considering that *Ae. japonicus* inhabits areas with abundant vegetation, being more abundant in rural settings (Bartlett-Healy et al., 2012), this study focuses on i) evaluating the association of the presence of AIM with factors related to the type of environment, urbanization degree, and population density and ii) investigating the range of distribution of *Ae. japonicus* in the Basque Country.

IV.4.2. Materials and methods

IV.4.2.1. Study area

The Basque Country is a small region in northern Spain with *ca*. 7200 km^2 divided into three administrative provinces: Gipuzkoa, Bizkaia and Araba. The average annual temperature and rainfall are 13.4 °C and 1,610 mm in Gipuzkoa, 13.8 °C and 1,278 mm in Bizkaia, and 11.5 °C and 878 mm in Araba. The population of the Basque Country is *ca.* 2,188,017 inhabitants, and industry and tourism are some of the driving forces of the Basque economy (EUSTAT, 2020). There is a network of highly trafficked motorways structured around the main routes that connect the Basque Country with France, central Spain, and the Mediterranean coast, facilitating the introduction of AIM to new areas by passive transportation.

IV.4.2.2. Sampling approach

The sampling strategy to monitor AIM presence in 2021 encompassed the placement of ovitraps in 45 municipalities with more than 10,000 inhabitants. Two sampling zones were selected in each municipality, except for the three main cities (Bilbao, Donostia-San Sebastian and Vitoria-Gasteiz), where the number of selected zones increased to 8, 17, and 4, respectively. A total of 113 sampling areas were selected.

Fieldwork was carried out by a network of health officers from the Public Health Directorate of the Basque Government and from the municipalities of Bilbao, Donostia-San Sebastian, Vitoria-Gasteiz, and Laguardia. Sampling started on June 1st and finished on November 18th. Thus, each municipality and area were sampled from 11 to 12 times over a period of 23 weeks (June-November).

IV.4.2.3. Sampling methodology

The presence of *Aedes* spp. eggs was investigated using oviposition traps (ovitraps) (ECDC, 2012). A total of 568 ovitraps were deployed (Gipuzkoa: 275 ovitraps, 19 municipalities, 55 sampling areas; Bizkaia: 235 ovitraps, 22 municipalities, 47 sampling areas; Araba: 58 ovitraps, 4 municipalities, 11 sampling areas) (Table 17). Five ovitraps were placed in each sampling area, always in shady places and hidden in the vegetation. Each ovitrap contained dechlorinated water and a wooden stick (masonite; 12 cm long and 2.5 cm width) as oviposition substrate. Oviposition sticks were removed and replaced by new sticks every 15 days.

Information on each sampling area was compiled, including geographic coordinates and type of environment (parking, green park, petrol station, city center, industrial zone). Sampling sites were also categorized according to the urbanization degree: urban, suburban, and peri-urban areas. Based on a previous work (Loibl et al., 2011) and adjusted to the socio-geographical reality of the region, the sampling areas were categorized as follows: Urban areas were those in the town center, including urban core and inner urban areas; suburban areas were mainly residential, not densely compacted, and located near an inner urban area; peri-urban areas were spaces located on the boundaries of the town with scarce urban development, and including both urban-fringe and urban periphery.

IV.4.2.4. Laboratorial methods

Oviposition sticks were examined in the laboratory under a stereomicroscope (zoom magnification range of 7.5 – 135 x). If eggs were observed, they were counted, and positive sticks were preserved for egg hatching by immersion in a Petri dish with dechlorinated water. Emerged larvae were placed in mosquito breeders (Bioquip ®, Compton, CA, USA) at room temperature (ca. 23 °C) until becoming adult mosquitoes. The identification of *Aedes* mosquitoes was performed using taxonomic keys (Becker et al., 2020). In the case of unsuccessful egg hatching, 5-10 eggs were collected from selected sticks, and DNA extraction was performed using a commercial kit (NZY Tissue gDNA isolation kit, NZYtech, Lisboa, Portugal), followed by PCR targeting the COI gene (Delgado-Serra et al., 2021; Simon et al., 1994). Amplicons were sequenced by external services using the Sanger technique. Sequences were compared with those available in GenBank by BLAST analysis to confirm the species. A selection of sequences is deposited in GenBank with the reference numbers OQ884140-OQ884148.

IV.4.2.5. Statistical analyses

The positive ovitrap index (POI) [(number of positive traps/number of inspected traps) x 100] was estimated for each sampling area (Nascimento et al., 2020). A sampling area was considered positive when the presence of *Aedes* spp. eggs was detected in at least one oviposition trap in at least one sampling. To represent the overall egg-laying kinetics of AIMs over the sampling period (week 24 to week 46), the means of eggs counted each fortnight were calculated and represented in a graph with a smoothed trend line based on LOESS (locally estimated scatterplot smoothing). Associations between the presence of *Ae. albopictus* and *Ae. japonicus* (as a dependent binomial variable) with province, urbanization (urban/suburban/peri-urban) and type of environment (town, petrol station, parking, green parks and industrial areas) were analyzed using logistic regression models (GLM), and odds ratios (OR) were calculated using the ORci function of the CIplot package. Censuses from each municipality included in the study were compiled. Thus, the presence of each species according to the human population density of the municipality (inh/km^2) was evaluated by the Wilcoxon rank-sum test. All statistical analyses were performed using R statistical software version 3.6.1 (R Core Team, 2022).

IV.4.3. Results

IV.4.3.1. Distribution of invasive mosquitoes

A total of 5,896 oviposition sticks were examined in 2021 (2,853 from Gipuzkoa, 2,484 from Bizkaia; 559 from Araba). Eggs of *Aedes* spp. were detected in 66.4% of the sampling sites, and 32.4% of the ovitraps were distributed in the three territories of the Basque Country (Table 17). In 18 sampling sites from 13 municipalities, only one positive ovitrap and one oviposition stick were positive throughout the surveillance period. In contrast, in 10 sampling sites from 6 municipalities, the five ovitraps harbored *Aedes* eggs during more than 8 samplings. The province of Gipuzkoa showed a higher percentage of positive ovitraps (42.5%) than Bizkaia (27.7%) and Araba (3.4%). Likewise, the percentage of oviposition sticks with eggs was higher in Gipuzkoa (12.2% *vs*. 5.0% in Bizkaia and 0.4% in Araba) (Table 17).

Table 17. Number of municipalities, sampling areas, ovitraps and oviposition sticks examined in the three Basque provinces and prevalence of eggs of *Aedes* spp.

		Sampling areas		**Ovitraps**		**Oviposition sticks examined**	
Province	**No. municipalities**	**No.**	**Posit. *Aedes* eggs (%)**	**No.**	**Posit. *Aedes* eggs (%)**	**No.**	**Posit. *Aedes* eggs (%)**
Araba	4	11	1 (9.1)	58	2 (3.4)	559	2 (0.4)
Bizkaia	22	47	31 (66.0)	235	65 (27.7)	2,484	124 (5.0)
Gipuzkoa	19	55	43 (78.2)	275	117 (42.5)	2,853	349 (12.2)
Total	45	113	75 (66.4)	568	184 (32.4)	5,896	475 (8.1)

It is noteworthy that 100% of the municipalities of Gipuzkoa were positive for the presence of *Aedes* spp., while in Araba, *Aedes* eggs were detected only in one of the four municipalities investigated (Table 18).

Table 18. Municipalities included in the surveillance programme and the number of them harboring AIM, *Aedes albopictus, Aedes japonicus* or both species simultaneously.

Province	No. municipalities	Total with AIM (% pos)	*Ae. albopictus* (% pos)	*Ae. japonicus* (% pos)	*Ae. albopictus* and *Ae. japonicus* (% pos)
Araba	4	1 (25.0)	0	0	1 (25.0)
Bizkaia	22	18 (81.8)	6 (27.3)	5 (22.7)	7 (31.8)
Gipuzkoa	19	19 (100)	6 (31.6)	10 (52.6)	3 (15.8)
Total	45	38 (84.4)	12 (26.7)	15 (33.3)	11 (24.4)

The establishment of *Aedes* spp. according to POI, was confirmed in several municipalities (Figure 22A). The most intense red color indicates that between 75 and 100% of the ovitraps of the municipality were positive for the presence of *Aedes* eggs.

IV.4.3.2. Identification of *Aedes* spp.

To identify the species of *Aedes* in each sampling site, a total of 195 oviposition sticks with eggs were submitted to molting to obtain adult mosquitoes that were identified by morphological keys, as described above. When adult emergence failed, PCR of the eggs and sequencing were carried out. The species of *Aedes* was known in 40.2% of the positive oviposition sticks (191/475, 69 from Bizkaia, 120 from Gipuzkoa, 2 from Araba), confirming the presence of *Ae. albopictus* in 44 sampling areas from 23 municipalities, and *Ae. japonicus* in 39 sampling areas from 26 municipalities (Table 18; Figure 22B).

Aedes albopictus was concentrated around large urban centers such as Bilbao and Donostia/San Sebastian, where the population density is higher and industrialized areas are abundant in the surrounding areas. *Aedes japonicus* predominated in less populated areas (Figure 22B). Concurrence of both species was observed in eleven municipalities (Table 18). Both *Ae. japonicus* and *Ae. albopictus* were simultaneously present on the same oviposition stick on just one occasion. Eggs from *Aedes geniculatus* (Olivier, 1971) were identified by molecular methods in only one oviposition stick. No other *Aedes* species were identified by morphological or molecular methods.

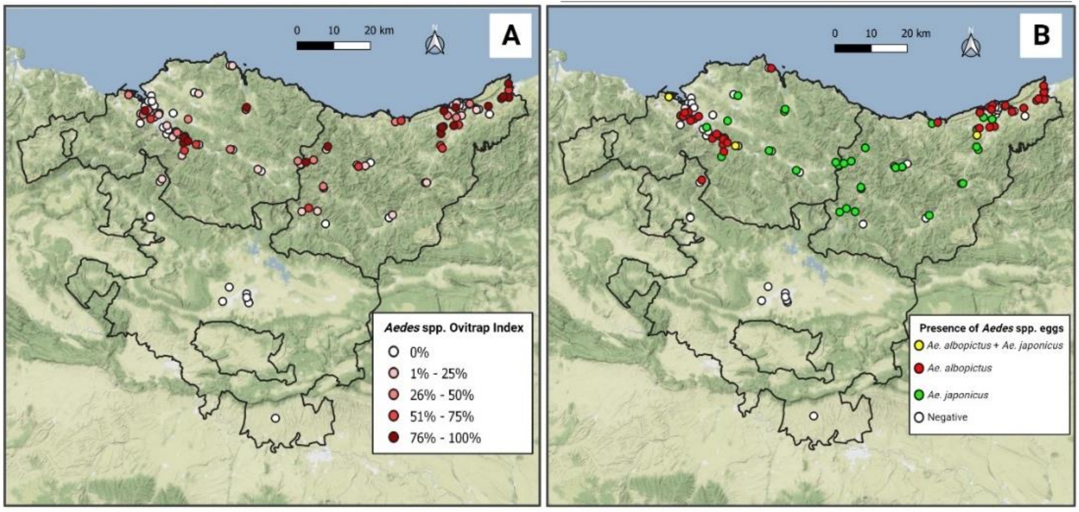

Figure 22. Distribution of *Aedes* spp. eggs in each sampling site according to the Ovitrap Index, represented in a gradient of color (A). Distribution of *Aedes albopictus* (red dots), *Aedes japonicus* (green dots) and coexistence of both species (yellow dots) (B).

IV.4.3.3. Oviposition activity of *Aedes* spp.

Aedes egg-laying activity progressively increased throughout the sampling period, with a maximum between September and November. *Aedes* egg laying was significantly higher in the province of Gipuzkoa than in Bizkaia and Araba (Kruskal–Wallis $\chi^2 = 230.02$, $P < 0.0001$) (Figure 23A). Considering the twelve municipalities where only *Ae. albopictus* was identified, egg laying peaked between September and October (Figure 23B). Similarly, taking into consideration the fifteen municipalities with the only detection of *Ae. japonicus*, egg-laying activity showed a more stable pattern throughout the study period, with an earlier peak in July (Figure 23C).

IV.4.3.4. Distribution of AIM by urbanization degree and characteristics of the environment

The probability of *Ae. albopictus* occurrence was higher in Bizkaia (OR= 14.62) and Gipuzkoa (OR=18.36) than in Araba*,* while that for *Ae. japonicus* was significantly high in Gipuzkoa (OR= 11.40) (Table 19). Interestingly, the presence of *Ae. albopictus* was associated with municipalities with a higher population density (mean= 2,983 inh/km^2) ($P < 0.001$), and the presence of *Ae. japonicus* was associated with lower population density (mean= 1,590 inh/km^2) ($P < 0.05$) (Figure 24).

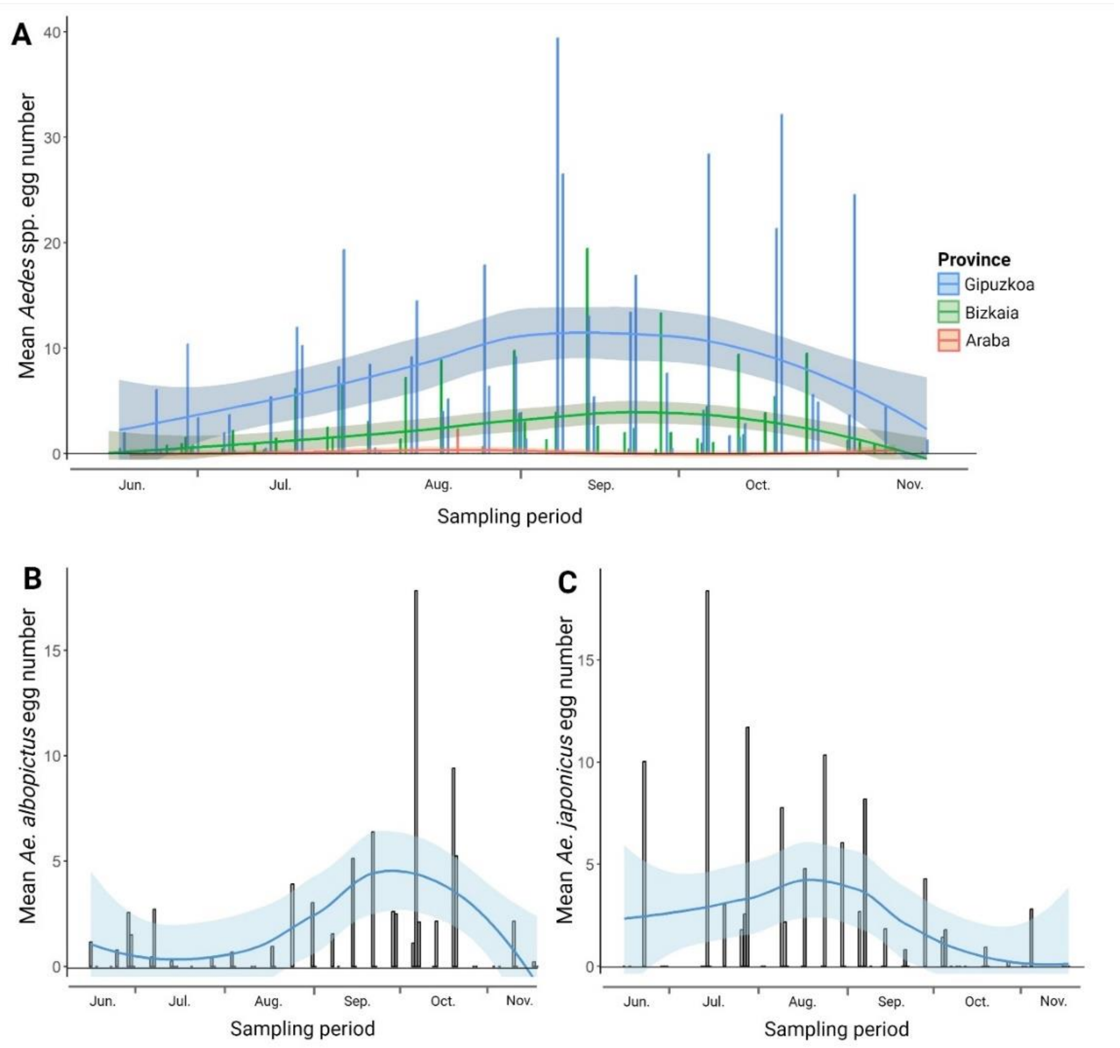

Figure 23. Mean *Aedes* spp. egg laying in the three Basque provinces (A). *Aedes albopictus* (B) and *Aedes japonicus* egg laying (C) considering only the municipalities where each species was exclusively found (12 and 15, respectively).

The analysis between the presence of AIM and the degree of urbanization (urban, suburban, peri-urban) revealed that, whereas *Ae. albopictus* showed a 4.39 times higher probability of being found in suburban areas (p = 0.001) than in peri-urban areas, *Ae. japonicus* had a higher probability of being found in peri-urban areas than in suburban or urban areas (p = 0.001) (Table 19; Figure 24).

Considering the characteristics of the environment, the distribution of both species relied on the type of site. Thus, the probability of finding *Ae. albopictus* was significantly higher in areas surrounding parking lots ($P < 0.05$), while the probability of finding *Ae. japonicus* was higher in areas surrounding petrol stations and industrial areas ($P < 0.05$) (Table 19).

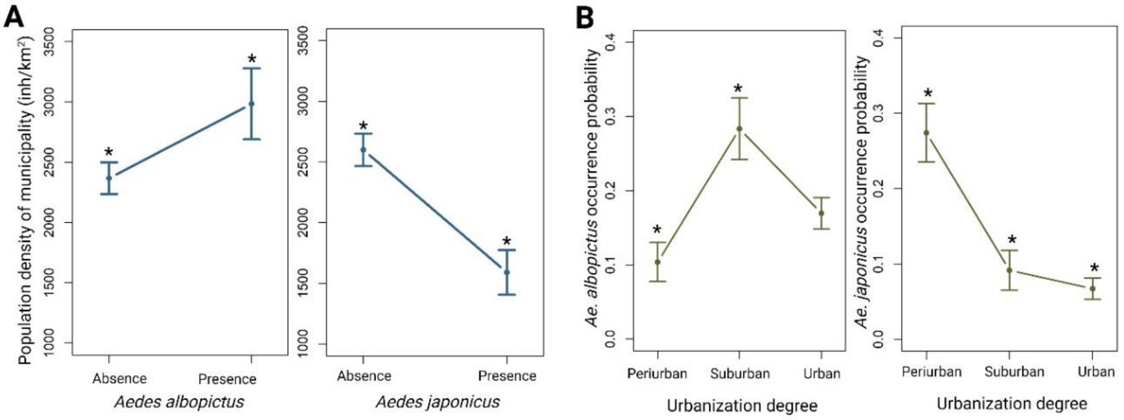

Figure 24. Presence of each species according to the population density (inh/km^2) (A); occurrence probability of *Aedes albopictus* and *Aedes japonicus* according to the urbanization gradient (B).

IV.4.4. Discussion

The successful establishment of invasive mosquito populations in new environments is a complex process influenced by several factors, including climate, habitat, and human activity (Medlock, et al., 2012b). Six invasive species have been established in Europe (Giunti et al., 2023a; Miranda et al., 2022), and among them, *Ae. albopictus* and *Ae. japonicus* are becoming widespread (Giunti et al., 2023a; Koban et al., 2019). The wide distribution observed in the Basque Country (northern Spain) confirms the spread and establishment of these species of invasive mosquitoes in the region. Our results demonstrate that, *Ae. albopictus* predominated around the two main cities (Bilbao and San Sebastián) that harbor a larger population per km^2, more traffic and important industrial ring roads, which favoring the introduction and distribution of this species (Lucientes-Curdi et al., 2014; Swan et al., 2022). The province of Gipuzkoa was the probable point of entry of *Ae. albopictus* in the Basque Country (Goiri et al., 2020), and since then, significantly higher activity has been detected in this province, as shown in the current study. Until recently, the AIM surveillance program in this region was focused on placing ovitraps in areas with high concentrations of traffic, which favored the detection of *Ae. albopictus* (Goiri et al., 2020). In 2020, the surveillance was extended to municipalities that were less populated and less industrialized, enabling the detection of *Ae. japonicus* in four municipalities (Miranda et al., 2022). Furthermore, the sampling strategy carried out in the present study (2021), which encompassed urban, suburban, and peri-urban areas, showed a wide distribution of *Ae.*

japonicus in the studied region. It is difficult to know how long this species has been part of the entomofauna of the Basque Country and the possible route of entry. However, in the two years since its first detection (Eritja et al., 2021), established populations of *Ae. japonicus* have been detected in a higher number of municipalities than *Ae. albopictus*.

Table 19. Summary of logistic regression models for *Aedes albopictus* and *Aedes japonicus* regarding province, urbanization degree and type of environment.

Variables	*Aedes albopictus*			*Aedes japonicus*		
	E ± SE[1]	**OR[2] (95% CI)[3]**	**p-value**	**E ± SE**	**OR (95% CI)**	**p-value**
Province						
Araba	Ref.[4]			Ref.		
Bizkaia	2.68±1.02	14.62 (3.01- 263.69)	0.009	1.42±1.06	4.15 (0.77-77.33)	0.180
Gipuzkoa	2.91±1.02	18.36 (3.82-330.28)	0.004	2.43±1.03	11.40 (2.30-207.13)	0.018*
Urbanization degree						
Periurban	Ref.			Ref.		
Suburban	1.48±0.35	4.39 (2.22-9.11)	0.001*	-1.24±0.39	0.28 (0.12-0.59)	0.001*
Urban	0.95±0.36	2.60 (1.30-5.48)	0.008*	-1.18±0.36	0.30 (0.14-0.62)	0.001*
Type of environment						
Green park	Ref.			Ref.		
Petrol station/ Industrial area	0.42±0.44	1.53 (0.63-3.65)	0.33	0.93±0.46	2.55 (1.047-6.46)	0.041*
Housing	0.02±0.30	1.02 (0.56-1.86)	0.94	-0.06±0.41	0.93 (0.40-2.13)	0.86
Parking	0.61±0.30	1.84 (1.01-3.39)	0.047*	0.32±0.41	1.38 (0.61-3.15)	0.42

[1] Est ± SE = Estimate ± Standard Error; [2] OR = Odds ratio; [3] 95% CI =95% confidence intervals; [4] Ref. = reference category. * p-value < 0.001

In contrast to *Ae. albopictus*, which is distributed by passive dispersal through motorized vehicles, *Ae. japonicus* seems to be distributed over time by active dispersal (Müller et al., 2020), with an estimation of spreading more than 100 km within a few years (Kaufman and Fonseca, 2014; Montarsi et al., 2019). Thus, in countries such as Hungary or Italy, *Ae. japonicus* spread rapidly in a short period by appropriate ecological corridors, finding natural and artificial containers to breed (Montarsi et al., 2019; Sáringer-Kenyeres et al., 2020). Considering that, in our study, the occurrence probability of *Ae. japonicus* is greater in Gipuzkoa (province bordering France), it could be speculated that this species may have come from France. However, *Ae. japonicus* seem to be established only in northeastern France (ECDC, 2023c), and there are no reports in southern France bordering our study area. It is important to highlight that, as this study demonstrates, if AIM surveillance is focused only on urban areas, the presence and distribution of *Ae. japonicus* may be underestimated.

Climatic and demographic variables, such as temperature, precipitation, and population density, are key factors in mosquito distribution (Brugueras et al., 2020). In this study, a widespread presence of both species was observed in northern regions with a mesothermal Atlantic climate (moderate temperatures and extensive rainfall); however, no specimens were detected in the southern area with a transitional climate toward the Mediterranean (drier and warmer summers). *Aedes albopictus* has strong ecological plasticity, can be established in a wide range of different habitats with different climatic conditions and is better adapted to warmer climates than *Ae. japonicus* (Giunti et al., 2023a). These could be the reasons for the establishment of *Ae. japonicus* in regions of Spain with temperate Atlantic climates, such as northern Spain (Eritja et al., 2021). In addition, larvae of *Ae. japonicus* do not tolerate water temperatures above 30 °C (Andreadis and Wolfe, 2010), which occur during the summer in central and southern Spain.

In northern Italy, which enjoys colder winters and snow and relatively warm summers, the seasonal activity period of *Ae. japonicus* lasts at least seven months between April and November (Montarsi et al., 2019). However, in the study area, with milder winters, larvae were found in February and March in man-made containers around farmhouses in the provinces of Bizkaia and Gipuzkoa (unpublished data). This study also indicates that egg laying starts earlier for *Ae. japonicus* than *Ae. albopictus*. In fact, the predictions of species distribution according to the habitat suitability under climate change are different for both AIM species; whereas *Ae. albopictus* is promoted by climate change, the area modeled to be climatically suitable for *Ae. japonicus* is projected to decrease in Europe (Cunze et al., 2016) as it would not be able to adapt to warmer climatic conditions.

In addition, landscape structure is key to facilitating the occurrence of *Ae. japonicus,* even in a climatically unsuitable region, and *vice versa* (Kerkow et al., 2020). In general, the presence of *Ae. japonicus* is more prevalent in vegetation-rich (Chaves et al., 2020; Kampen and Werner, 2014) and rural areas compared to urban and suburban areas (Bartlett-Healy et al., 2012), or in the transition zones between forest and settlements (Früh et al., 2020). This study also showed a higher probability of finding *Ae. japonicus* in petrol stations and industrial areas. Both emplacements are usually surrounded by shrubs and vegetation-rich areas. Moreover, these areas offer optimal resting places for mosquitoes in addition to a wide variety of artificial breeding containers. In contrast, the probability of finding *Ae. albopictus* has been higher in parking lots, which may be due to the role of terrestrial vehicles enabling the passive dispersion of this species (Eritja et al., 2017; Ibáñez-Justicia, 2020).

Urbanization processes modify the environment, have a major impact on the mosquito species community, and lead to biodiversity loss caused by anthropogenic changes (Ferraguti et al., 2016). The more urbanized a given area is, the fewer species are found, but mosquito species adapted to urban environments increase in abundance (Wilke, et al., 2021b). This is especially applicable to container-breeding and invasive mosquitoes (Wilke et al., 2019, 2020a). In the current study, the presence of *Ae. albopictus* and *Ae. japonicus* varied depending on the degree of urbanization. Thus, *Ae. albopictus* appeared concentrated in urban and suburban areas, probably due to the higher availability and density of artificial breeding sites, its tolerance to hotter and drier climate conditions, and the presence of a wider blood-feeding host range, including humans (Li et al., 2014; Mogi et al., 2020). In fact, this mosquito species is considered strongly anthropophilic, and in urban areas where the human population is greater, a higher blood-feeding rate has been observed (Valerio et al., 2010). In this study, *Ae. albopictus* showed a higher presence in municipalities with higher population densities. In contrast, *Ae. japonicus* showed a preference for municipalities with lower population density, such as peri-urban environments and rural settings (Mogi et al., 2020). Urban areas are occasionally colonized by *Ae. japonicus*, but hotter and drier summer conditions caused by the effect known as 'urban heat island" (LaDeau et al., 2015), would negatively impact its life cycle. In addition, a higher variety of available mammal hosts to feed on could be the reason that they prefer peri-urban and rural settings (Mogi et al., 2020). Although the two species seem to coexist without much evidence of displacement, the potential competitive interaction between the larval stages of the two species should not be ignored and should be further investigated.

This study confirms a new colonization area in Europe for *Ae. japonicus*. Even though *Ae. japonicus* is not considered a high-risk mosquito for public health, it is unknown whether the role of the species could change if its distribution and abundance increase. In addition, *Ae. albopictus* is expected to be fully established in the coming years, causing nuisance (Goiri et al., 2020) and increasing the likelihood of autochthonous arbovirus transmission. The present results may have practical applications for the design of AIM surveillance. Due to the potential impact on public health and according to our findings, surveillance programs should be designed considering different types of environments, including municipalities with low population density and peri-urban areas. Mosquito surveillance and control activities will continue in the study area to keep population density at minimum. Special attention will be paid on the characterization of the *Aedes* breeding sites to better understand the distribution pattern of these two species of invasive mosquitoes.

V. General Discussion

Mosquitoes are not only annoying biters, but are vectors of important diseases such as malaria, dengue, Zika and chikungunya, among others (Weaver et al., 2018). Consequently, it is important to know which mosquito species are present in an area, as well as their abundance, to evaluate risks of emergence of these diseases (ECDC & EFSA, 2018). In fact, the expanded distribution of exotic and native mosquitoes is causing an increase in the incidence of vector-borne diseases, especially in previously free areas (Lühken et al., 2023). Moreover, it is crucial to investigate mosquitoes in different types of environments to know species present and their dynamics (Ferraguti et al., 2023; Wilke et al., 2017). This is because mosquitoes are very adaptable and can thrive in a wide range of habitats (Dale & Knight, 2008). Therefore, conducting systematic mosquito sampling across diverse environments allows to identify which species colonize specific habitats as well as to determine which are predominant. This information is essential for understanding their ecological preferences and niches.

The knowledge on the diversity, abundance, and distribution of mosquitoes in the Basque Country before this thesis was scarce. The main objective of this thesis was to broaden this knowledge by sampling and identifying culicids in different types of environments of the three Basque provinces. To know which mosquito species are present, as well as their distribution, abundance, and ecological preferences in different habitats it is necessary to use as many trapping techniques as possible (ECDC, 2014, 2018). In this thesis, Study I demonstrate that combination of CDC traps with dipping and sweep netting has allowed the detection of several species not detected by a single trapping method. Thus, we observed that netting was more effective for collecting *Cx. territans* and *Ae. detritus*, while CDC-traps were more effective for collecting *Cx. modestus* and *An. claviger* s.l.. Furthermore, male mosquito catches were significantly higher using sweep nets than CDC traps, as well as bloodfed mosquitoes. In addition, the dipping method allowed the identification of several species, such as *Cx. hortensis* (Study I), *Cs. longiareolata* (Study I) or *Cx. territans* (Study III), in sampling areas where adults were not catched by CDC traps. Dipping is an interesting technique to complement the adult sampling methods, as it can determine the abundance of the immature stages, and the type of breeding habitat (ECDC, 2018).

In addition, with the use of HLC technique, we have achieved preliminary results on the blood-feeding behaviour of annoying species such as *Ae. rusticus, Ae. detritus, Ae. cantans*, *Ae. caspius*, *Cx. modestus*, *An. claviger, and Cx. pipiens* s.l. Therefore, the HLC method could be incorporated to the routine methods to know better the impact of Culicidae on biting nuisance to humans, as recommended by other authors (ECDC, 2018; Uelmen et al., 2023). However, it is important to consider the ethic aspects because of the risk for the operator to become infected by pathogens. Moreover, HLC may not be a good indicator of anthropophilic preferences of certain mosquito species, as their abundance in the environment has to be also considered (Cansado-Utrilla et al., 2020; Kenea et al., 2017; Maliti et al., 2015).

Furthermore, it would also be of interest to include the use of the BG Sentinel traps in the mosquito samplings as they are particularly designed for the capture of specific species, such as AIM (ECDC, 2012, 2018). In fact, it is interesting to note that very few AIM were captured by CDC traps, even after the observation of the establishment of *Ae. albopictus* and *Ae. japonicus* in many municipalities of our region, as seen in Study IV.

Mosquito species composition in the Basque Country

Before the start of the thesis, in the Basque Country 12 different species including *Cx. pipiens* s.l., *Culex. impudicus*, *Cx. territans*, *An. maculipennis* s.s., *Ae. caspius*, *Ae. detritus*, *Cx. mimeticus*, *Cs. subochrea*, *Cx. hortensis*, *Cs. longiareolata*, *Cs. litorea*, and the exotic mosquito *Ae. albopictus* had been reported (Bueno-Marí et al., 2012; Cirujano et al., 2003; de Castro, 2004; Delacour et al., 2015; González et al., 2015). Most of these reports are from natural areas in the province of Gipuzkoa, except the studies of Cirujano et al. (2003) and González et al. (2015) which took place in natural and urban environments in the city of Vitoria-Gasteiz (Araba) respectively. Regarding the province of Bizkaia, as far as we know, no records of culicid species were available. However, *Cx. pipiens* s.l. was considered to be present in all the provinces of Spain (Bueno-Marí et al., 2012), thus being the only culicid potentially present in Bizkaia.

Other studies carried out in parallel to this thesis investigated the resting places of Culicidae in an urban-natural gradient using an aspirator, identifying new records for the Basque Country, such as *An. claviger s.l., Cs. fumipennis, Cs. morsitans, Cx. theileri* and *Ur. ungiculata* (González et al., 2020). In addition, the investigation of mosquito breeding sites led to identify *Ae. cantans*, *Ae. vexans* and *Cx. torrentium* (González et al., 2021). Besides, *Ae. rusticus* and *Cx. modestus* were found in golf courses in Araba (González et al., 2022). Also, a second AIM species, *Ae. japonicus*, was identified (Eritja et al., 2021; González et al., 2022).

Interestingly, two of the above-mentioned mosquito species (*Cx. impudicus* and *Ur. unguiculata*) (Cirujano et al., 2003; González et al., 2020) have not been detected in this thesis. *Uranotaenia unguiculata* is widely distributed in the Mediterranean region (Robert et al., 2019). In Spain, it has been reported in northern regions of Navarra and La Rioja in natural environments (Ruiz-Arrondo et al., 2019). It is a species difficult to collect using conventional trapping methods as CO_2 baited light traps (Camp et al., 2018), thus explaining the lack of catches in the samplings done in this thesis. Moreover, Ruiz-Arrondo et al. (2019), also stated that it may be easier to capture this species while resting. This could explain why González et al. (2020) were able to catch this species, using an aspirator. As mentioned above, *Cx. impudicus* was reported in the study of Cirujano et al. (2003), that took place in the wetland of Salburua, but because of the difficulty to differentiate *Cx. impudicus* and *Cx. territans* in the larva stage (Becker et al., 2020), as done by Cirujano et al. (2003), this identification might be in discussion. Moreover, in the Study I of this thesis, carried out in the weltland of Salburua during two consecutive years, only *Cx. territans* was identified by morphological and molecular methods. However, in Spain, it is considered a native species (Robert et al., 2019), and it has been found near the Basque Country, in La Rioja (Bueno-Marí et al., 2012; Ruiz-Arrondo et al., 2019).

In this thesis, five new records are reported for the first time in the region (*Ae. geniculatus*, *Ae. sticticus*, *An. atroparvus*, *Cq. buxtoni,* and *Cq. richiardii*).

Table 20 summarises the 30 mosquito species identified in the Basque Country, belonging to six different genera. Among them, two invasive species, *Ae. albopictus* and *Ae. japonicus*, widely distributed in the area, as seen in Study IV.

Moreover, in this thesis different mosquito community composition has been observed in the three Basque provinces, being the provinces of Bizkaia and Gipuzkoa more similar in comparison with Araba (Figure 25). This result is not surprising, because Bizkaia and Gipuzkoa provinces enjoy the Atlantic climate while Araba is continental and has a transitional climate between Atlantic and Mediterranean (Euskalmet, 2022). Araba has the highest species richness of the three Basque provinces with 21 different species. In Bizkaia 20 mosquito species have been identified while in Gipuzkoa a total of 15 species of mosquito have been identified so far (Figure 25). However, it has to be taken into account that in the province of Gipuzkoa no natural areas were sampled, and therefore the diversity of species in this territory would be probably higher. Nevertheless, as the provinces of Gipuzkoa and Bizkaia are very similar orographically and climatically, having both Atlantic climates, it is very likely that the species already identified in the natural area of Bizkaia are also present in that type of environment in the province of Gipuzkoa. On the other hand, in all the provinces, the two IMS detected in the Basque Country are present but with different abundance, as shown in Study IV.

Table 20. Culicidae identified in this thesis, and in other studies carried out in the Basque Country. In **bold** are indicated the new citations reported in this thesis for the Basque Country.

Genus	Species	Reference	Other citations in the Basque Country
Aedes	*Ae. albopictus*	This thesis	4, 6
	Ae. cantans	This thesis	9, 10
	Ae. caspius	This thesis	1
	Ae. detritus	This thesis	1
	Ae. geniculatus	**This thesis**	
	Ae. japonicus	This thesis	5, 10
	Ae. rusticus	This thesis	10
	Ae. sticticus	**This thesis**	
	Ae. vexans	This thesis	9
Anopheles	*An. claviger s.l.*	This thesis	8, 9, 10
	An. atroparvus	**This thesis**	
	An.maculipennis s.s.	This thesis	1, 8, 9
	An. plumbeus	This thesis	8, 9, 10
Coquillettidia	*Cq. buxtoni*	**This thesis**	
	Cq. richiardii	**This thesis**	
Culiseta	*Cs. annulata*	This thesis	8, 9, 10
	Cs. fumipennis	This thesis	8
	Cs. litorea	This thesis	7, 8
	Cs. longiareolata	This thesis	4, 7, 8, 9, 10
	Cs. morsitans	This thesis	8
	Cs. subochrea	This thesis	1
Culex	*Cx. impudicus*		2
	Cx. hortensis	This thesis	4, 7, 8, 9, 10
	Cx. mimeticus	This thesis	1, 9
	Cx. modestus	This thesis	10
	Cx. pipiens s.l.	This thesis	2, 4, 7, 8, 9, 10
	Cx. territans	This thesis	3, 8, 9, 10
	Cx. theileri	This thesis	8, 9
	Cx. torrentium	This thesis	9, 10
Uranotaenia	*Ur. unguiculata*		8

1, Bueno-Marí et al., 2012; 2, Cirujano et al., 2003; 3, de Castro et al., 2004; 4, Delacour et al., 2015; 5, Eritja et al., 2021; 6, Goiri et al., 2020; 7, González et al., 2015; 8, González et al., 2020; 9, González et al., 2021;10, González et al., 2022

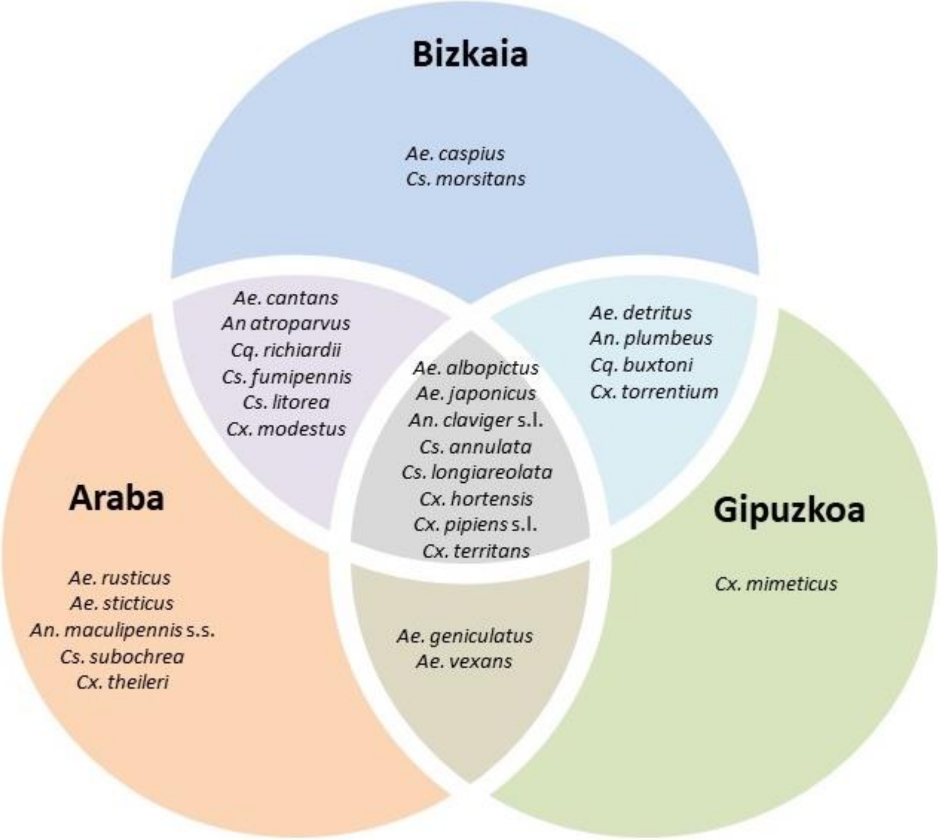

Figure 25. Mosquito species identified in this thesis in each Basque province.

Taking together all the results obtained in the different studies of the thesis, as well as with the rest of the studies done in the Basque Country, a total of 30 species were identified (28 native and 2 invasive). In the province of Araba there is a total of 26 species of mosquitoes, also considering *Ur. unguiculata* (González et al., 2020), *Cx. impudicus* (Cirujano et al., 2003), as well as *An. plumbeus, Cx. mimeticus* and *Cx. torrentium* (González et al., 2021). Likewise, in Gipuzkoa there would be a total of 17 species, adding *Ae. caspius* and *An. maculipennis* s.s. previously identified (Bueno-Marí et al., 2012) to the species found in the thesis. Finally, in Bizkaia, 20 species were identified (Figure 25).

Regarding the province of Araba, five mosquito species, including *Ae. rusticus*, *Ae. sticticus*, *An. maculipennis* s.s., *Cs. subochrea*, and *Cx. theileri*, were present only in this territory. *Aedes rusticus* is usually found in wet woodlands subject to periodic flooding (Medlock & Vaux, 2015b) and has a wide distribution around Europe (Robert et al., 2019). In this thesis *Ae. rusticus* was found in the natural and urban green areas, but interestingly this species was catched by CDC traps and sweep netting, and despite its abundance in the wetland of Salburua, no larvae were observed in the breeding sites examined around traps (Table 21). This could be because our samplings started in May, and we detected the highest peak of adult mosquitoes' activity at the end of the spring season (May-June). Therefore, as it is an univoltine species, finding its larvae after the adult emergence could be difficult (Becker et al., 2020; González, 2022; Hawkes et al., 2020). Another *Aedes* spp. mosquito only found in the province of Araba is *Ae. sticticus*, which is a predominant floodwater mosquito found in river valley areas (Becker et al., 2020; Schäfer and Lundström, 2009). It usually has an abundance peak which occurs after the floods and hatches in large numbers causing nuisance in these areas (Schäfer and Lundström, 2009, 2006). This species is widely distributed in Europe, being recorded from northern regions down to the Mediterranean area. In Spain, its distribution is scarce or still not well known (Bueno-Marí et al., 2012). In this thesis it has been found in low numbers in the natural area of Salburua, and, recently, has also been described in the wetlands of the nearby province of La Rioja (Ruiz-Arrondo et al., 2020) and reported as well in the province of Aragon (Ruiz-Arrondo et al., 2010).

Anopheles maculipennis s.s., was also identified in our study in the province of Araba and it was previously reported in a study by González et al. (2020), in a natural environment of this same province. Even though, this species is considered widespread in Europe, but it is described to be less common in the southern parts of the Iberian Peninsula (Bravo-Barriga et al., 2021, 2017; Taheri et al., 2024). Despite the small number of catches recorded in this thesis, this species could be more abundant in northern Spain, which has a similar climate to Central Europe with a higher humidity. On the other hand, this species has been reported to being expanding to northeastern Europe and northwestern Asia triggered by global warming and displacing other species belonging to the Maculipennis complex (Novikov and Vaulin, 2014). Therefore, it is interesting to study the distribution and abundance of the *An. maculipennis* s.l. species, both in Spain (Taheri et al., 2024) and Europe, to assess their displacement and the risk it could entitle in the expansion of Malaria through the continent. The low number of specimens captured in the different habitats in comparison to other *Culicidae,* hampers to extract any conclusion about the contribution of other sampling methods (Studies I and III) in the capture of larvae/ adults of *An. maculipennis* s.l.

Two specimens of *Culiseta subochrea* were found in the province of Araba, one in the Study I and the other in the Study III. It has to be taken into account, that some species within the *Culiseta* genus (*Cs. litorea*, *Cs. annulata* and *Cs. subochrea*) have been morphologically misidentified in the past years, due to different identification characters (Ruiz-Arrondo et al., 2020). In fact, *Cs subochrea* varies from *Cs. annulata* in a few characters (Becker et al., 2020), making them difficult to differentiate when the specimens are damaged. Moreover, these two species can be found breeding together (Becker et al., 2020), thus complicating the matter even more as they share the same type of habitat. For the correct differentiation of these two species, when doubtful or damaged specimens were found in this thesis, COI barcoding was used, and the sequences were compared to those deposited in the GenBank and BOLD databases. As seen in the Studies I, II and III, *Cs. annulata* is much more abundant in the Basque Country in comparison to *Cs. subochrea*, and was collected by both, CDC traps and dipping (Table 21). In fact, *Cs. annulata* was recorded in both aquatic environments sampled, in both green urban areas and cemeteries of the coastal and inland cities, and in APC of the inland city. Moreover, *Cs. annulata* was more abundant in natural environments than in urban areas. However, in the urban green urban areas it was the third most trapped culicid, which can be explained as this mosquito is able to b(Becker et al., 2020; Snow & Medlock, 2006; Townroe & Callaghan, 2014)&(Becker et al., 2020; Snow & Medlock, 2006; Townroe & Callaghan, 2014). *Culiseta litorea* was only found in the natural environments (Study I). This species can be confused morphologically with its siblings *Cs. morsitans* and *Cs. fumipennis* also due to similar diagnostic characteristics (Becker et al., 2020), thus, molecular techniques were used in this thesis for their identification. However, *Cs. litorea* is usually found breeding in sunlit pools and small ponds and ditches (Becker et al., 2020). Moreover, it is usually a coastal species as it can tolerate high saline waters (Becker et al., 2020), but as seen in the Study I of this thesis, it was found in both the Atlantic and continental wetlands.

Culex theileri was only found in the province of Araba both in the natural environment, and in the urban areas. This mosquito species is very adaptable and breeding sites can be found in different types of habitats from flooded fields in rural environments, to exploding man-made containers (Şimşek, 2004). Therefore, this adaptability explains the presence of this species in these two different environments, however, no larvae were catched by dipping in Studies I and III, although were previously found in puddles, standing pool waters, or backwater from urban areas (González et al., 2021) from the same province. Moreover, it has been found naturally infected with *Dirofilaria immitis* in the Island of Madeira (Portugal) (Santa-Ana et al., 2006), thus monitoring its population and dynamics is important for the control of this disease.

Aedes caspius and *Cs. morsitans* have been only detected in Bizkaia. *Aedes caspius* was trapped in the natural area (Urdaibai) and sporadically in the green area of Bilbao. *Aedes caspius* is a halophilic and common species in Atlantic and Mediterranean coastal marshes and rock holes (Becker et al., 2020). This species usually breeds in flooded areas where the level of water fluctuates frequently (Hawkes et al., 2020; Ruiz-Arrondo et al., 2023), although no larvae were found in larval habitats, nor in previous studies (González et al., 2021). *Culiseta morsitans* was only detected in the marsh of Urdaibai (Study I), showing its suitability to breed in that environment, being able to tolerate high saline and brackish water (Becker et al., 2020; Hawkes et al., 2020).

Another typical marsh and estuarine mosquito is *Ae. detritus*, commonly known as the salt-marsh mosquito (Hawkes et al., 2020), which has been also found in the marsh of Urdaibai and in the green area of Donostia. It is, as well as *Ae. caspius*, a common mosquito found in aquatic natural environments (Hawkes et al., 2020), however as stated in study III the green urban area of Donostia is a very naturalized park near the sea, thus explaining its presence there.

Among the new records for the Basque Country *Aedes geniculatus* is included, which is a common mosquito in Europe, being found in deciduous or mixed forests (ECDC, 2023a), where it lays its eggs in tree holes and open tree stumps (Müller et al., 2012). Although it has been described to rarely enter into urban environments (ECDC, 2023a), in this thesis, this species was only captured in two naturalised urban green areas. In addition to collecting adult mosquitoes by using CDC traps, larvae were found in tree holes. In Spain, it has been described in several provinces (Bueno-Marí et al., 2012), including the northern region of La Rioja (Ruiz-Arrondo et al., 2020).

Anopheles atroparvus also constitutes a new description for the Basque Country. This species is widely distributed throughout Europe (Robert et al., 2019), and in Spain (Bueno-Marí et al., 2012; Santa-Olalla Peralta et al., 2010; Taheri et al., 2024). It was considered the potential vector of the first autochthonous malaria outbreak in Spain since the eradication of the disease (Santa-Olalla Peralta et al., 2010). A few specimens of *An. atroparvus* were captured by CDC traps in the green urban area of Vitoria-Gasteiz and in the natural environment of Urdaibai. In Spain it can be frequently found in river margins, irrigation channels and small and temporary lagoons (Bueno-Marí and Jiménez-Peydró, 2012). However, in a study recently performed in the province of Araba to investigate larval breeding sites, *An. atroparvus* could not be found in its larval stage (González et al., 2021).

Coquillettidia buxtoni were identified for the first time in the Basque Country in the provinces of Gipuzkoa and Bizkaia. Due to its limited detection up until 2015, *Cq. buxtoni* was considered a rare finding in Europe (Becker et al., 2020). Since the start of the VectorNet project (Robert et al., 2019) this species has been found present in Spain, France, Germany, Switzerland, and Italy, among other regions of the western Palearctic. In Spain, *Cq. buxtoni* has been identified in Galicia (Martínez-Barciela et al., 2021), Girona, Tarragona, and Castellon (Bueno-Marí et al., 2012). In Galicia, the larvae of *Cq. buxtoni* were in continental and coastal lagoons sharing habitat with other culicid species such as *Cx. theileri*, *An. maculipennis* s.l. and *Cs. annulata* (Martínez-Barciela et al., 2021). Moreover, the lagoons had presence of plants such as *Potamogeton* sp. and *Juncus* sp. (Martínez-Barciela et al., 2021) favouring the breeding of this species. Therefore, to capture and obtain *Coquillettidia* larvae, its sampling has to be thorough and intense (Bueno-Marí, 2010; Romanowski & Candeletti, 1984). However, in previous studies done in the Basque country (González et al., 2021) larvae of *Cq. buxtoni* were not found in the breeding sites despite examining appropriate plants (*Phytotelmata* environments).

Only two *Coquillettidia* species are found in Europe, being *Cq. richiardii* the second species within this genus, which is widely distributed (Robert et al., 2019). In Spain, it had been found present in six provinces including Huelva, Salamanca, Teruel, Tarragona, Barcelona, and Girona (Bueno-Marí et al., 2012), and more recently in Valencia (Rueda et al., 2017, 2020), La Rioja (Ruiz-Arrondo et al., 2019, 2017b) and Galicia (Martínez-Barciela et al., 2021). In the Basque Country it has been detected in the provinces of Araba and Bizkaia, mainly in natural areas, specifically wetlands by CDC traps and dipping, being an abundant species in these environments (Rueda et al., 2017; Ruiz-Arrondo, Hernández-Triana, et al., 2017). However, *Cq. richiardii* can also be found in aquatic environments in rural, peri-urban (Ruiz-Arrondo et al., 2023) and urban areas (Lebl et al., 2015).

It is worth mentioning *An. plumbeus* among the species identified in the different environments of the Basque Country due to its potential sanitary interest because is an aggressive biter and has an anthropophilic behaviour (Dekoninck et al., 2011; Ibañez-Justicia & Cianci, 2015). Moreover, it is considered to be a potential vector of malaria in Europe (Bueno-Marí & Jiménez-Peydró, 2011a; Krüger et al., 2001; Schaffner et al., 2012). *Anopheles plumbeus* has a wide distribution around Europe b(Ibañez-Justicia & Cianci, 2015)therlands (Ibañez-Justicia & Cianci, 2015), Germany (Heym et al., 2017) or Belgium (Dekoninck et al., 2011). In Spain, *An. plumbeus* has been detected in around a dozen provinces (Bueno-Marí et al., 2012; Martínez-Barciela et al., 2020; Ruiz-Arrondo et al., 2019), to which may be added the provinces of Bizkaia and Gipuzkoa. This mosquito species has adapted and shifted its usual habitat from natural and forested areas to urban areas (Dekoninck et al., 2011; Schaffner et al., 2012) which matches the current results having found this species in urban and natural areas. This species has adapted from laying its eggs in the water surface on the side of a tree hole (Bueno-Marí & Jiménez-Peydró, 2011a), to exploiting man-made containers, therefore bringing this vector closer to human settlements (Schaffner et al., 2012). However, it has not been found in the larval breeding sites examined in Studies I and III

Table 21. *Culicidae* collected in this thesis by CDC traps and by dipping (D).

Culicidae	**Green urban areas**			**Cemeteries**			**APC**			**Natural areas**	
	Bilbao (BI)	**Donostia (GI)**	**Gasteiz (AR)**	**Bilbao (BI)**	**Donostia (GI)**	**Gasteiz (AR)**	**APC2 (BI)**	**APC3 (GI)**	**APC1 (AR)**	**Salburua (AR)**	**Urdaibai (BI)**
Ae. albopictus				■							
Ae. cantans										■	
Ae. caspius	■										■
Ae. detritus		■									■ D
Ae. geniculatus		■ D	■								
Ae. rusticus			■							■	
Ae. sticticus										■	
Ae. vexans		■	■							■	
An. claviger s.l.			■ D			■		■	■	■ D	■ D
An. atroparvus			■								■
An. maculipennis s.s.			■						■	■	
An. plumbeus		■			■		■				■
Cq. richiardii									■	■	■ D
Cq. buxtoni		■		■							■
Cs. annulata		■ D	■ D		■	■			■	■ D	■ D
Cs. fumipennis										■	■
Cs. litorea										■	■
Cs. longiareolata	■	■ D	■ D	■	■ D	■	■	■	■	D	
Cs. morsitans											■
Cs. subochrea			■							■	
Cx. hortensis				■	■ D	■					D
Cx. mimeticus											
Cx. modestus	■									■	■
Cx. pipiens s.l.	■ D	■ D	■ D	■ D	■ D	■	■	■	■	■ D	■ D
Cx. territans		D	■							D	■ D
Cx. theileri			■			■				■	
Cx. torrentium	D		D	■ D	■ D		■		■		■

APC: only the results from the CDC traps are included as breeding sites were not studied. When the shade of the box is lighter, it indicates that larvae have only been detected by dipping. BI: Bizkaia; GI: Gipuzkoa; AR: Araba

Only one specimen of *Cx. mimeticus* has been detected in Gipuzkoa. This mosquito species is easy to distinguish from the other *Culex* species, as they have spotted wings, that resemble some of the *Anopheles* genus (Becker et al., 2020; Somboon et al., 2023). It is usually found in many countries in mountainous or plain areas (Becker et al., 2020; Bueno-Marí & Jiménez-Peydró, 2011b) of the southern Palearctic region, including the Mediterranean area (Becker et al., 2020; Somboon et al., 2023). In Spain, it has been described in several regions (Bueno-Marí et al., 2012). Interestingly, this mosquito species exhibits a clear preference for wild environments (Bueno-Marí & Jiménez-Peydró, 2011b), being found in natural areas from the nearby provinces of La Rioja and Navarra (Ruiz-Arrondo et al., 2019, 2017b). Therefore, the presence in this thesis of *Cx. mimeticus* in a highly naturalized urban green area is quite interesting. The medical importance of this mosquito is low, but some authors have listed *Cx. mimeticus* as a potential vector of WNV (Ruiz-Arrondo et al., 2019). Therefore, its surveillance in urban areas could be important, even though no cases of this disease have been reported yet in northern Spain.

On the other hand, several species of culicids were found in all the provinces of the Basque Country (Figure 25). Among them, *Culex territans* is widely distributed having collected adult mosquitoes and larval stages, in all the type of environments studied. In Spain has been detected in 21 provinces in nine regions and in the Canary Islands (Bueno-Marí et al., 2012), in the Basque Country it was identified in the province of Gipuzkoa (de Castro, 2004), and it has been recently found in Galicia (Martínez-Barciela et al., 2021). It is known as the *northern frog-biting mosquito* (Knight & Stone, 1977), as it usually feeds on cold-blooded animals such as amphibians and reptiles (Bartlett-Healy et al., 2008; Eubanks et al., 2008). Therefore, it is usually found in swamps and water bodies where these cold-blooded animals are present and this species is considered as an indicator, together with the anurans, of the quality of the water as these species do not tolerate even low levels of pollution (Crans, 2023). It has also been found in urban areas with artificial surfaces in Germany (Krüger et al., 2014). In this study, the wetland of Salburua is an example of the perfect habitat for this mosquito species, as it hosts a wide range of amphibians and reptiles to feed on (Cirujano et al., 2003). Moreover, it was also observed that *Cx. territans* is not very attracted to adult traps but can be easily found in their breeding sites (Krüger et al., 2014). This has been clearly seen in Studies I and III in some urban green areas and natural areas, where this species has only been detected in its larval form (Table 21). It is also interesting to note that despite being a species that is easily identifiable in the breeding sites of the Basque Country (González et al., 2021), the types of containers that are usually found in cemeteries are not suitable for this species of mosquito to breed.

Culex hortensis is widely distributed in the Mediterranean region. In Spain is present in nearly all the regions including the Canary Islands (Bueno-Marí et al., 2012). Moreover, this species was identified in natural and urban environments (Bueno Marí and Jiménez-Peydró, 2011b). Similar to *Cx. territans*, *Cx. hortensis* feeds mainly on amphibians and reptiles (Schaffner et al., 2010). In this thesis, *Cx. hortensis* was a scarce mosquito in natural spaces as a few specimens were captured in the form of larvae (28) and adult (1) using dipping and sweep netting respectively, as seen in Study I. However, adult *Cx. hortensis* were captured by CDC traps in the cemeteries of the three cities investigated. This is interesting because, considering that *Cx. hortensis* is a culicid difficult to collect with CO_2-baited CDC light traps (Roiz et al., 2012), it suggests that it is an abundant species in cemeteries. Moreover, a lot of larvae from this species were collected in the cemetery of Gipuzkoa, as vases and flowerpots were filled with water with certain amount of vegetation or algae, which is suitable for their development (Becker et al., 2020; Schaffner et al., 2010).

Regarding *Cs. longiareolata*, adult mosquitoes have been identified in all types of environments, except in natural spaces, where only two larvae were identified in the wetland of Salburua. However, in a previous study of larval breeding sites of Culicidae (González et al., 2021), *Cs. longiareolata* was also detected in natural and rural habitats. Larvae can be found in rock holes but can also colonize artificial containers (Becker et al., 2020; Townroe and Callaghan, 2014). It is the second most abundant species in all the green urban environments sampled, only being surpassed by the ubiquitous mosquito *Cx. pipiens* s.l. This species is widely distributed in the Mediterranean Region (Ferraguti et al., 2023), being described in several provinces of Spain (Bueno-Marí et al., 2012), including the Basque Country for the first time in 2014 (Delacour et al., 2015). In the Northern countries of Europe, it is considered an invasive species that has been colonizing new territories due to global change (Becker and Hoffmann, 2011; Deblauwe et al., 2021; Seidel et al., 2013). It is mainly an ornithophilic species that rarely feeds on humans or enter human dwellings (Becker et al., 2020).

The species belonging to the *Cx. pipiens* complex were the most abundant species collected in all the different environments sampled, except in the natural habitat, where other Culicidae predominated. The *Pipiens* complex consists of several species, subspecies, and ecoforms, that are difficult to morphologically differentiate, specifically the females (Becker et al., 2020). *Culex pipiens* s.l. is a species belonging to the complex and is the most common mosquito species in Europe (Brugman et al., 2018). In Spain, it is considered to be present in all the Spanish regions (Bueno-Marí et al., 2012). This species has three different ecoforms: *Culex pipiens* form *pipiens*, *Culex pipiens* form *molestus* and their hybrid *Culex pipiens pipiens*/*molestus* (Haba and McBride, 2022). Each ecoform is adapted to specific ecological niches and habitats, then having behavioural and physiological differences (Brugman et al., 2018; Haba and McBride, 2022). The *pipiens* form is associated with natural and rural environments, while the *molestus* ecoform is described as being well adapted to urban habitats (Brugman et al., 2018; Vinogradova, 2000). However, these ecological properties are not so rigid, and *Cx. pipiens pipiens* can be also found in urban environments (Haba and McBride, 2022) In this study the most abundant ecological form in natural areas was *Cx. pipiens pipiens* (Table 22), whereas in some urban areas or cemeteries the proportions of both ecoforms, *Cx. pipiens molestus* and *Cx. pipiens pipiens,* were similar (Table 22).

Table 22. Summary of the ecoforms of *Culex pipiens* s.l. found in this thesis.

Location	**Study - Description**	**No. *Cx. pipiens* s.l. analysed**	**No. *Cx. pipiens pipiens* (%)**	**No. *Cx. pipiens molestus* (%)**	**No. *Cx. pipiens* hybrid (%)**
Araba - wetland	I - wetland	15*	13 (86.7)	1 (6.7)	1 (6.7)
Bizkaia - wetland	I - wetland	7*	6 (85.7)	1 (14.3)	0
Araba - APC	II - APC1 peri-urban	12*	12 (100)	0	0
Bizkaia - APC	II - APC2 peri-urban	4*	3 (75.0)	1 (25.0)	0
Gipuzkoa - APC	II - APC3 rural	4*	4 (100)	0	0
Araba - inland city	III - urban green area	16	11 (68.8)	5 (31.2)	0
Bizkaia - estuarine city	III - urban green area-park	62	34 (54.8)	17 (27.4)	11 (17.7)
Gipuzkoa - coastal city	III - urban green area-park	31	14 (45.2)	15 (48.4)	2 (6.4)
Araba - inland city	III - cemetery	11	8 (72.7)	1 (9.1)	2 (18.2)
Bizkaia - estuarine city	III - cemetery	12	5 (41.7)	6 (50.0)	1 (8.3)
Gipuzkoa - coastal city	III - cemetery	14	10 (71.4)	2 (14.3)	2 (14.3)
Total		**188**	**120 (63.8)**	**49 (26.1)**	**19 (10.1)**

*Only damaged mosquitoes were analysed (Studies I and II)

However, the best characterization of *Cx. pipiens* forms has been carried out in green zones and cemeteries (ca. 25% of *Cx. pipiens* analysed), since in Studies I and II only doubtful or damaged specimens were identified. Thus, in this latter case, the results cannot be definitive, and a larger number of specimens should be investigated to get a more representative picture of the predominant ecoforms of *Cx. pipiens* in the natural areas and in places with presence of animals (in rural and periurban areas) in order to confirm which Cx. *pipiens* s.l. ecoform is more prevalent in each environment. In our study, the *pipiens/molestus* hybrids have been detected in the green urban areas and cemeteries in proportions between 12% (13/109) and 13% (5/37), respectively. Interestingly, the percentage of hybrids recorded in the region of Extremadura (25.7%) is higher in comparison to the findings of this thesis (Bravo-Barriga et al., 2017). Moreover, different studies have suggested that hybridization between *pipiens* and *molestus* could produce bridge vectors which may result in outbreaks of WNV in large urban areas (Fonseca et al., 2004; Huang et al., 2009; Kilpatrick et al., 2007). Therefore, it is important to determine the rate of *Cx. pipiens* s.l. ecoforms in different regions across Spain and Europe to assess the risk of this WNV transmission, which may increase in northern areas of Europe with the rise of the temperature (Farooq et al., 2023; Vogels et al., 2017b). The dynamics of *Culex pipiens* ecoforms have a direct impact on disease transmission, therefore understanding which ecoform is prevalent in a given area can provide insights into the risk of diseases such as WN and filariasis. For example, *Culex pipiens molestus*, often found in urban areas, may have a more significant role in the transmission of diseases that affect humans, given their close proximity to potential hosts. Additionally, variations in ecoform behaviour can influence the timing and intensity of disease outbreaks (Vogels et al., 2017b).

Another species belonging to the *Pipiens* complex is *Cx. torrentium*. This species is widespread across Europe (Becker et al., 2020). Moreover, *Cx. torrentium and Cx. pipiens* s.l. occur in sympatry in most of Europe, with the proportion of both species differing also in a latitudinal gradient (Weitzel et al., 2011). In fact, *Cx. torrentium* dominates in northern Europe while *Cx. pipiens* s.l. is more common in the south, with the proportion of both species being similar in Central Europe (Hesson et al., 2014; Lühken et al., 2015; Weitzel et al., 2011). In Spain, *Cx. torrentium* has been detected in northern regions (Bueno-Marí et al., 2012; Martínez-Barciela et al., 2020). Very few specimens of *Cx. torrentium* (adult mosquitoes and/or larvae) were identified in Studies I, II and III as they morphologically resemble its sibling species *Cx. pipiens* s.l., being only the male adult morphologically distinguished (Becker et al., 2020). Therefore, its identification relies on the use of molecular methods (Brugman et al., 2018). In the Basque Country, this species has also been found breeding in natural and rural environments in small artificial containers (González et al., 2021). Moreover, this species has been described to be expanding their range to northern regions of Spain (Ruiz-Arrondo et al., 2020), thus our finding of these species in our region, supports this assumption.

Invasive *Aedes* species have rapidly spread worldwide mainly through the international transport of goods by air, ship and even by car (Eritja et al., 2017; Ibáñez-Justicia, 2020). Of the six different *Aedes* invasive species detected so far in Europe (Giunti et al., 2023a), in our region we have so far recorded two of them: *Ae. albopictus* and *Ae. japonicus*. Currently, *Ae. albopictus* is well established in the provinces of Bizkaia and Gipuzkoa thriving in urban environments. The point of entry of the *Ae. albopictus* species is clearer, as it was detected for the first time in Gipuzkoa, in a municipality near the place where tiger mosquito was found in the South of France (Goiri et al., 2020). Moreover, the expansion of *Ae. albopictus* in our region started in

2014 being slow and progressive, and starting to be a nuisance in some municipalities in 2018 (Goiri et al., 2020), until being completely established as seen in Study IV. Moreover, *Ae. japonicus* is greatly expanded in the region, even though it is difficult to know when this mosquito species arrived in our region. In fact, it was surprising after its first detection in Asturias in 2018 (Eritja et al., 2019), how many municipalities of the Basque Country have established populations of *Ae. japonicus*. Moreover, the point of entry of this species in the Basque Country is still unknown, as curiously the higher populations are reported in the province of Gipuzkoa, but the previous closest findings of this species were in the Spanish provinces of Asturias and Cantabria, which are closer to the province of Bizkaia. Another highlight from Study IV is the need to monitor as well periurban and suburban areas, as the presence and distribution of *Ae. japonicus* could be underestimated.

Seasonal activity, abundance and diversity of mosquito species

Objective I also aimed at identifying the factors affecting mosquito presence, abundance, and richness in different type of environments. Mosquitoes' seasonal dynamics affect their population densities during the year. These peaks of abundance are not yet fully understood and differ between mosquito species (Becker et al., 2020). Moreover, understanding the mosquito population dynamics can help determining the timing of the peak abundance, nuisance, and risk of disease transmission (Altizer et al., 2006; Ewing et al., 2019). These seasonal dynamics are driven by different factors including environmental conditions, such as temperature, humidity, and the availability of breeding sites (Kilpatrick and Randolph, 2012). Hence, in the Study I the peak of overall abundance in the natural area of Salburua was the earliest peak registered in our studies driven by the emergence of the snowmelt mosquito *Ae. rusticus*. This is an univoltine mosquito usually found in wet woodlands and hatch in early spring (Medlock & Vaux, 2015b). In contrast, the overall abundance in the other natural area (Urdaibai) showed a bimodal pattern being explained by a first emergence of *Coquillettidia* spp. in July and followed by a smaller peak of activity of *Cx. modestus*. On the other hand, in urban and peri-urban areas (Studies II and III) the seasonal pattern was more complex exhibiting a sinusoidal trend. In those areas, the most collected species was *Cx. pipiens* s.l., which is a multivoltine species (Becker et al., 2020). These multiple generations per year are conditioned by climatic factors, thus explaining their population dynamics which vary inter and intra-annually (Ewing et al., 2016). Regarding AIM, *Ae. japonicus* showed an earlier peak of abundance (early spring) in comparison to *Ae. albopictus* whose higher abundance was reported at the end of the summer (Study IV). This is due because these two *Aedes* species have different habitat and climatic requirements, as *Ae. japonicus* prefers milder environments while *Ae. albopictus* can tolerate warmer conditions (Cunze et al., 2016). Interestingly, according to Cunze et al. (2016) there are very few areas of Europe, where these two species can overlap.

Furthermore, mosquito abundance and species richness vary across different type of environments. In our studies, natural areas (Study I) hosted the higher abundance in all the environments sampled, followed by urban areas (Study III), and APCs (Study II) (Table 24). In fact, the lowest species richness was observed in the APC of Gipuzkoa, followed by the APC and green urban area of Bilbao. On the other hand, the highest species richness was reported in the natural areas, as expected (Ferraguti et al., 2016, 2022) (Table 23). It is interesting to mention, the high species richness found in the green areas of Vitoria-Gasteiz and Donostia, but as it was already discussed in Study III, these two areas are highly naturalised, thus being able to host more species (Perrin et al., 2022). However, the green urban area of Bilbao, as well as the APCs and cemeteries, are cemented areas with scarce vegetation mainly consisting of patches of grass and trees. On the other hand, the lowest Shannon diversity index (H’) was recorded in the green area of Bilbao, this is also an expected finding, as landscape anthropization decreases the availability of breeding habitats, thus lowering the diversity of species present in urban areas (Ferraguti et al., 2022; Perrin et al., 2022). Moreover, the diversity index observed in the APCs was similar to the one reported in the green area of Bilbao, and in two of the three cemeteries sampled (Table 23), due to its cemented characteristics already mentioned. On the other hand, the highest diversity was observed in the natural environments, followed by the green area of Vitoria-Gasteiz closely and, finally both urban areas of Donostia. The higher diversity reported in these urban environments is because these areas have more vegetation and therefore can host a wider range of mosquito species, as seen in Study III.

This result raises important questions about the ecological and environmental factors contributing to the differences observed between type of environments. The factors affecting mosquito distribution and abundance are highly connected, as they play a key role in creating suitable breeding habitats for different mosquito species. For example, ecological and environmental conditions, like temperature and humidity influence the reproductive cycle, as well as their survival, which then impacts their overall abundance (Ferraccioli et al., 2023; Tahir et al., 2023), but unfortunately, this has not been investigated in this thesis. Furthermore, the type of environment and land-use, provides different characteristics for the breeding habitats of mosquito species, such as water availability, and cover vegetation (Ruairuen et al., 2022), which then influences the diversity of the species present in that given area, as observed in Study I. For the comparison of the factors affecting mosquito abundance, distribution, seasonality, richness, and diversity, we used GLM approaches comparing differences between locations, month of sampling, year, and type of sampling area, when it applied. In this thesis it was observed that the peak of abundance varied significantly from month to month, depending on the type of sampled area and the presence of different mosquito species, whether univoltine or multivoltine (Ferraccioli et al., 2023; Mora-Rubio et al., 2023), as demonstrated in Study I, where abundance was higher in July, decreasing slowly the following months with lowest abundance recorded in October. However, in the urban environments (Study III), the peak of abundance was found to be significantly higher in the month of August, contrasting with the earlier studies where the peak occurred in July. Additionally, interannual variations were noted, highlighting the influence of the year as a factor affecting both abundance and diversity (Mora-Rubio et al., 2023; Poh et al., 2019), as evidenced in Study III. Regarding the differences in the mean abundance between the type of sampling areas (natural vs. periurban vs. urban), our findings revealed a distinct pattern of mosquito abundance distribution. The highest abundance was consistently recorded in the two natural environments, underscoring their significance as thriving habitats for mosquitoes. Following this, areas exhibited a notable but comparatively lower abundance, highlighting the role of vegetation in influencing mosquito populations. Cemeteries, while still contributing to overall mosquito abundance, demonstrated a further decline in comparison. Finally, the least abundance was observed in APCs, emphasizing the importance of considering specific environmental characteristics in understanding mosquito distribution and abundance dynamics (Table 24).

Table 23. Species richness (S) and Shannon diversity Index (H') of the different type of habitats studied.

Studies	**Areas**	**Diversity index**	
		Species Richness (S)	**Shannon Index (H')**
Green urban areas	Bilbao	4	0.41
	Donostia	10	1.09
	Vitoria	13	1.61
Cemeteries	Bilbao	6	0.72
	Donostia	6	1.11
	Vitoria	6	0.73
APC	APC2	4	0.74
	APC3	3	0.60
	APC1	6	0.78
Natural areas	Salburua	17	1.83
	Urdaibai	17	2.09

In summary, and according to the findings of this thesis, landscape anthropization is a key factor contributing to the abundance and distribution of mosquito species (Perrin et al., 2022). Natural areas, like wetlands and forests, also known as “untouched environments”, tend to have higher mosquito abundance and species richness than human-altered environments because of their lower influence in these types of areas (Dale and Knight, 2008; Möhlmann et al., 2017). For example, in Spain, a study by Roiz et al. (2015) found that in Mediterranean wetlands the modification of the landscape by humans affected the abundance, distribution and species richness of mosquitoes. These findings are in accordance with the results presented in this thesis. This is because natural areas provide a wide range of breeding habitats (e.g. ponds and swamps) for the development of mosquitoes, as well as a wide range of ecological niches, thus supporting different mosquito species (Martínez-Barciela et al., 2024) and also higher availability of hosts for the mosquitoes to feed on (Burkett-Cadena et al., 2013).

The most abundant species differed between geographical locations with *Cq. richiardii* being more abundant in the marsh of Urdaibai, while *Ae. rusticus* was more abundant in the wetland of Salburua. Moreover, higher mosquito biodiversity in these environments also contributes to their overall abundance (Perrin et al., 2022). Nonetheless, in addition to the favourable conditions that natural environments have for a higher diversity of mosquito species (Wilke et al., 2021a), it should be noted that Study I used a wide range of sampling techniques, which allowed for an excellent picture of the Culicidae present in these natural areas.

The anthropization effect can be observed in this thesis, as the abundance and species richness of mosquitoes decrease from natural to urban environments (Table 23 and Table 24), as observed elsewhere (Ferraguti et al., 2016; Perrin et al., 2022). Urbanization is the process of altering the natural environment for the growth and accommodation of human expansion (Johnson and Munshi-South, 2017). Besides, Ferraguti et al. (2022), suggest that the alteration of the trophic interactions and the simplification of the habitats caused by humans could be the cause of the decreased biodiversity seen in urban environments. In this study, the most abundant native mosquitoes trapped in these environments have been *Cx. pipiens* s.l. and *Cs. longiareolata*. These two species are well-adapted to urban environments around the world (Ferraguti et al., 2023). Therefore, it is not surprising that these native species are the most abundant in green urban environments such as parks and cemeteries, as well as in peri-urban and suburban areas, as observed in Studies II and III.

Table 24. Mean abundance of each mosquito species per CDC trap and night in the different habitats investigated.

Culicidae	**Green urban areas**			**Cemeteries**			**APC***			**Natural areas**	
	Bilbao (BI)	**Donostia (GI)**	**Vitoria (AR)**	**Bilbao (BI)**	**Donostia (GI)**	**Bilbao (BI)**	**Donostia (GI)**	**Vitoria (AR)**	**Bilbao (BI)**	**Salburua (AR)**	**Bilbao (BI)**
Ae. albopictus				0.02							
Ae. cantans										1.36	
Ae. caspius	0.02										0.62
Ae. detritus		0.02									0,78
Ae. geniculatus		0.18	0.02								
Ae. rusticus			0.12							3,16	
Ae. sticticus										0,05	
Ae. vexans		0,02	0,08							0,02	
An. claviger s.l.			0,24			0,02		0,03	0,03	0,84	0,96
An. atroparvus			0,02								0,07
An. maculipennis s.s.			0,04						0,03	0,04	
An. plumbeus		0,16			0,06		0,16				0,02
Cq. richiardii									0,03	0,35	4,6
Cq. buxtoni		0,10		0,02							1,78
Cs. annulata		0,26	0,14		0,04	0,02			0,03	0,27	0,29
Cs. fumipennis										0,04	0,05
Cs. litorea										0,04	0,02
Cs. longiareolata	0,74	0,24	0,24	0,10	0,88	0,16	0,44	0,16	0,72		
Cs. morsitans											0,18
Cs. subochrea			0,02							0,02	
Cx. hortensis				0,06	0,12	0,02					
Cx. mimeticus		0,02									
Cx. modestus	0,02									0,02	2,55
Cx. pipiens s.l./ Cx. torrentium	5,34	2,88	1,30	1,00	0,96	0,94	1,91	0,75	2,47	1,29	1,71
Cx. territans			0,02								0,04
Cx. theileri			0,12			0,02				0,02	
Mean Abundance	**7.65**	**4.30**	**2.36**	**1.89**	**2.14**	**1.15**	**2.47**	**0.91**	**3.72**	**7.56**	**13.76**

Microhabitats also contribute to the diversity and abundance of mosquitoes in urban environments (Rhodes et al., 2022). The presence of vegetation together with microclimatic conditions and the availability of water provide the perfect conditions for the breeding and resting sites for the development and survival of mosquitoes (Erraguntla et al., 2021; Sauer et al., 2021). Water can be found in urban environments in lagoons, fountains, or water reservoirs such as drains (Crocker et al., 2017). However, small man-made containers such as cans, buckets etc., are beneficial for certain types of mosquitoes, such as *Aedes* invasive species where they thrive (Ibáñez-Justicia, 2020), which could explain the wide distribution of these exotic mosquitoes in the cities and towns of the Basque Country, as shown in Study IV. Moreover, in these microhabitats, other organisms that interact with the mosquitoes are present, which include competitors, predators, and hosts (Carlson et al., 2004; Shaalan and Canyon, 2009). These microhabitat properties found in urban areas can explain the different diversity of mosquito species found among cities.

Feeding preferences of native mosquitoes in the Basque Country

Regarding the objective III focused on the feedings patterns of mosquito species along an urban-to-natural gradient, we have obtained interesting results which complement the findings of a previous study done in the Basque Country around Vitoria-Gasteiz (González et al., 2020). In fact, host selection influences the exposure of mosquitoes to pathogens which then can become involved in the transmission of these diseases that can affect humans and animals (Yan et al., 2021). Therefore, knowing the host feeding preferences of mosquitoes is very important to understand the epidemic transmission pattern of MBD (Martínez de la Puente et al., 2021; Yan et al., 2021). Some mosquitoes have a preference toward a group of hosts (Eubanks et al., 2008), and others have an opportunistic feeding on the first available host (Martínez de la Puente et al., 2012). It is a complex phenomenon, thus deciphering these patterns in field-based studies is quite difficult as there are other factors involved, such as host defences (Estep et al., 2012), or the circadian cycle of the hosts and mosquitoes (Day and Edman, 1984). Moreover, the innate preference of the hosts is driven by the olfactory system of the mosquitoes (Robinson et al., 2018; Takken and Verhulst, 2013). In Studies I and III a total of 70 mosquitoes fed on 13 animal species have been analysed (Table 25), and host DNA identification has been achieved in 39 of them, which represents a success rate of 56%, similar to what has been found in other studies (Estrada-Franco et al., 2020; González et al., 2020). The DNA identified corresponded to the predominant fauna in each area, with, for example, ungulate DNA detected in both wetlands and different bird species in urban parks. It is interesting to note that human DNA was only detected on one occasion in *Cx. modestus*, a species that is quite aggressive towards humans

(Becker et al., 2020). Therefore, it is interesting to compare the hosts DNA identified, together with the mosquito species, in the different types of environments that we studied (Table 25). Thus, in the natural environments *Aedes* mosquitoes exclusively fed on mammals, together with *Coquillettidia* spp. On the other hand, *Cs. annulata* showed a more opportunistic behaviour feeding in birds and mammals. Regarding the *Culex* genus, they showed an ornithophilic preference together with *An. claviger* s.l. and *Cs. morsitans*. As mentioned earlier, the only species that we found feeding on humans was *Cx. modestus* (Table 25). Mosquito species exhibit distinct feeding preferences, particularly in the selection of their hosts (Börstler et al., 2016; Takken and Verhulst, 2013) but are also influenced by the availability and abundance of potential hosts in their environment (Takken and Verhulst, 2013; Yan et al., 2021). This is important because, for zoonotic transmission of arbovirus, the vector must show some degree of generalist preference to feed both in birds and humans (Campos et al., 2023; Takken and Verhulst, 2013). Thus, understanding the feeding preferences of species that are major vectors of diseases is vital for designing interventions to protect human health. In general, *Cx. pipiens* was the most blood-fed species trapped (Table 25) and its ornithophilic behaviour is well-studied in Europe (Brugman et al., 2017b; Gomes et al., 2013; Muñoz et al., 2012; Radrova et al., 2013; Roiz et al., 2012b) as well as in a previous study performed in our region (González et al., 2020). In the thesis, the affinity of *Cx. pipiens* as well as *Culiseta* to feed on birds has been corroborated. This is important because mosquito populations that mostly feed on birds, would have a higher capacity to amplify WNV in comparison to other mammals which do not support viremias high enough to infect mosquitoes (Platt et al., 2007). However, it also must be taken into account the importance of mammals as a relevant host as well (Alcaide et al., 2009; Börstler et al., 2016; Martínez-De La Puente et al., 2016; Muñoz et al., 2011; Rizzoli et al., 2015).

Further research is needed on the blood-feeding sources of native and invasive Culicidae, because at present blood-feeding in AIM has not been studied so far in our territory. Moreover, not many blood-fed specimens of native mosquitoes were analysed, thus it is difficult to make robust conclusions. In further studies, to better study the blood-feeding preferences, it is important to make aspiration of mosquitoes in resting places since it has been proven an effective trapping technique for blood-fed mosquitoes (Hernandez-Colina et al., 2021; Martínez de la Puente et al., 2020; González et al., 2020).

Table 25. Host DNA blood-meals determined in mosquito species from Study I and Study III.

Culicidae	N. analysed *	Area	Location	Host DNA	N.**
Ae. cantans	1	wetland	Salburua	*Cervus elaphus*	1
Ae. caspius	3	wetland	Urdaibai	*Sus scrofa*	1
Ae. detritus	9	wetland	Urdaibai	*Bos taurus*	6
				Ovis aries	2
Ae. rusticus	12	wetland	Salburua	*Cervus elaphus*	8
An. claviger s.l.	1	wetland	Salburua	*Meleagris gallopavo*	1
	2	wetland	Urdaibai	**no amplification**	
Cq. buxtoni	5	wetland	Urdaibai	*Sus scrofa*	1
Cq. richiardii	3	wetland	Urdaibai	*Equus caballus*	1
				Sus scrofa	1
Cx. modestus	3	wetland	Urdaibai	*Anas platyrhynchos*	1
				Turdus spp.	1
				Homo sapiens	1
Cx. pipiens s.l.	2	wetland	Salburua	*Parus major*	1
	1	wetland	Urdaibai	*Turdus philomelos*	1
	3	Green urban area	estuarine city	*Turdus merula*	1
	9	Green urban area	coastal city	*Turdus merula*	1
				Turdus philomelos	1
				Anas platyrhynchos	1
				Erithacus rubecula	1
				Serinus serinus	1
				Serinus canarius	1
				Passer domesticus	1
Cs. annulata	2	wetland	Salburua	*Meleagris gallopavo*	2
	6	wetland	Urdaibai	*Bos taurus*	1
	1	Green urban area	coastal city	**no amplification**	
Cs. litorea	1	wetland	Urdaibai	**no amplification**	
Cs. longiareolata	4	Green urban area	Coastal city	**no amplification**	
Cs. morsitans	2	wetland	Urdaibai	*Turdus merula*	1

* Number of mosquito specimens analysed for the identification of host DNA;
** Number of host DNA identified

Many autochthonous outbreaks of MBD have been detected in Europe, including Spain (Cochet et al., 2022; Lazzarini et al., 2020; Monge et al., 2020). Luckily, no autochthonous cases of MBD, like as dengue, chikungunya, Zika or West Nile (WN), have been reported in the Basque Country so far. The lack of WN, for example, in our region could be a result of the low abundance of native mosquitoes' main vectors of WN, such as *Culex* spp., as summarised in Table , in comparison to the ones registered in other Spanish regions (Roiz et al., 2012a), which have reported cases (García San Miguel Rodríguez-Alarcón et al., 2021; López-Ruiz et al., 2018). Besides, until the year 2021 IMS showed a progressive expansion in our region, but as stated in the Study IV of this thesis, they are completely established in certain municipalities. Moreover, as seen in the Study IV, municipalities with low density populations, as well as periurban areas could also be in danger of autochthonous outbreaks of dengue, Chickungunya and other arboviruses, as AIM species also find these environments suitable and thrive in them.

So far, there are 30 species of mosquitoes in the Basque Country, but this situation could change due to future changes in urban and rural planning or by reordering natural environments. Additionally, the number of mosquito species in the region may evolve with the conduct of new studies or the exploration of other landscapes, influencing the overall mosquito diversity and composition. Changes in land use could cause an increase in the interactions of vectors and hosts that could lead to the emergence or re-emergence of MBD (Ferraguti et al., 2022; Guo et al., 2019). In fact, the changes and movements made by humans are causing a new concerning scenario for Public Health in Europe (Ibáñez-Justicia, 2020). Therefore, the transformation of areas, either by landscape anthropization of natural and rural environments or by the creation of green areas in urban environments, must be performed with care, as these transformations could mean the increase of mosquito populations. As shown in Study IV, as well as in previous studies (Goiri et al. 2020) the involvement and cooperation of the staff from town councils, and the Departments of Public Health are necessary for an effective control and surveillance of invasive mosquito populations. This cooperation should be extended to native mosquito surveillance in urban, natural, and rural areas. In fact, the involvement of the community in eliminating potential breeding sites has been proven very important and effective in the control strategy of these vectors (Bonnet et al., 2020; Giunti et al., 2023a; Ibañez-Justicia et al., 2018). Therefore, the management of water resources as well as waste residues is also fundamental for the control of the vectors and their diseases. Moreover, the expansion of certain mosquito species with high adaptability and plasticity such as *Ae. albopictus* and *Cx. pipiens* in urban environments is guaranteed, as they will find the perfect climatic and environmental conditions for their development, as well as the availability of host to feed on (Garrido et al., 2024; Marini et al., 2017). In fact, mosquito diversity in these areas will probably decrease and these species will displace the native species previously present in these environments.

The thorough investigation of mosquitoes in various environmental settings is essential for the understanding of these vectors, as they will play a key role in the planning and management of urban environments in the near future. Therefore, researching mosquito ecology and their breeding behaviour in diverse habitats, gives important information to guide and help in the creation of resilient and sustainable urban landscapes. This knowledge can then be applied in the design and improvement of urban settings that can improve and resolve the nuisance that these dipterans create in green urban areas. Furthermore, by using this knowledge, control measures could be designed more effectively, developing more targeted and sustainable strategies that not only reduce mosquito-borne disease transmission but also have a positive environmental impact.

VI. Conclusions

1. In the Basque Country there is a wide variety of species of mosquitoes, with higher abundance in natural areas compared to urban, rural, or peri-urban zones. The predominance of one mosquito species over another varies according to the habitat; various species of *Aedes* or *Coquillettidia* prevail in natural areas, while *Culex pipiens* s.l. dominates in urban settings.
2. Mosquito sampling must be carried out using various techniques to thoroughly characterize the composition and abundance of the species present. In addition to the methods used in this thesis (CDC traps baited with CO_2, sweep netting, study of mosquito breeding sites, human landing collection), BG-Sentinel traps should be included to enhance the capture of exotic invasive species.
3. In presence of animals, CO_2 as bait in CDC traps does not offer any advantage in capturing more Culicidae. Besides, the fact that CDC traps with UV-light are more effective in capturing mosquitoes than CDC with white-light traps needs more research.
4. The seasonal kinetics of mosquitoes vary depending on the Culicidae species, whether univoltine or multivoltine. Abundance is influenced by habitat type, sampling month, and year.
5. Although the species richness of Culicidae is higher in natural areas, certain urban green spaces also exhibit considerable richness, depending on vegetation and landscape design, as observed in the plant nursery of Vitoria-Gasteiz or the park in Donostia.
6. The ecoform *Cx. pipiens pipiens* predominates in the Basque Country, followed by *Cx. pipiens molestus* and hybrids. However, in specific habitats, the *molestus* ecoform can reach the same proportion as the *pipiens pipiens* form.
7. *Aedes* spp. and *Coquillettidia* spp. primarily feed on ungulates, while *Culex* and *Culiseta* spp. feed primarily on birds but also on mammals. Although some mosquito species are specific in seeking a food source, the host DNA found from mosquito blood reflects the vertebrate species present in each ecosystem, natural or urban.
8. During the period 2018-2020, a small number of invasive *Aedes* were captured using CDC traps. However, the samplings in 2021 using ovitraps revealed that *Aedes albopictus* and *Aedes japonicus* are widespread in the Atlantic influence area. Furthermore, a new colonization area of *Ae. japonicus* in Europe has been confirmed.

9. The presence of invasive *Aedes* mosquitoes depends on the degree of urbanization (urban, suburban, peri-urban) and population density, emphasizing the need to plan surveillance programs and control plans taking into account these variables.
10. The contributions of this thesis have expanded the existing knowledge of native and invasive mosquito species in the Basque Country, with 30 species identified so far. Notable for their abundance and wide distribution are native species like *Cx. pipiens* s.l., due to its role as a vector of West Nile virus, and invasive species like *Aedes albopictus*, vector of several arboviruses.

VII. References

Abdullah, S.A., Hezri, A.A., 2008. From Forest Landscape to Agricultural Landscape in the Developing Tropical Country of Malaysia: Pattern, Process, and Their Significance on Policy. Environ Manage 42, 907–917.

Abe, M., McCall, P.J., Lenhart, A., Villegas, E., Kroeger, A., 2005. The Buen Pastor cemetery in Trujillo, Venezuela: measuring dengue vector output from a public area. Trop Med Int Health 10, 597–603.

Adhami, J., Reiter, P., 1998. Introduction and establishment of *Aedes (Stegomyia) albopictus* skuse (Diptera: Culicidae) in Albania. J Am Mosq Control Assoc 14, 340–343.

Aitken, T.H.G., 1954. The Culicidae of Sardinia and Corsica (Diptera). Bull Entomol Res 45, 437–494.

Alcaide, M., Rico, C., Ruiz, S., Soriguer, R., Muñoz, J., Figuerola, J., 2009. Disentangling Vector-Borne Transmission Networks: A Universal DNA Barcoding Method to Identify Vertebrate Hosts from Arthropod Bloodmeals. PLoS One 4, e7092.

Ali, S., Gugliemini, O., Harber, S., Harrison, A., Houle, L., Ivory, J., Kersten, S., Khan, R., Kim, J., LeBoa, C., Nez-Whitfield, E., O'Marr, J., Rothenberg, E., Segnitz, R.M., Sila, S., Verwillow, A., Vogt, M., Yang, A., Mordecai, E.A., 2017. Environmental and Social Change Drive the Explosive Emergence of Zika Virus in the Americas. PLoS Negl Trop Dis 11, e0005135.

Altizer, S., Dobson, A., Hosseini, P., Hudson, P., Pascual, M., Rohani, P., 2006. Seasonality and the dynamics of infectious diseases. Ecol Lett 9, 467–484.

Amerasinghe, F., Indrajith, N., Sci, T.A.-C.J., 1995, undefined, 1995. Physico-chemical characteristics of mosquito breeding habitats in an irrigation development area in Sri Lanka. Cey J Sci (Biol Sci) 24, 13–29.

Anderson, J.F., Main, A.J., Ferrandino, F.J., 2020. Horizontal and Vertical Transmission of West Nile Virus by *Aedes vexans* (Diptera: Culicidae). J Med Entomol 57, 1614–1618.

Andreadis, T.G., Wolfe, R.J., 2010. Evidence for Reduction of Native Mosquitoes with Increased Expansion of Invasive *Ochlerotatus japonicus japonicus* (Diptera: Culicidae) in the Northeastern United States. J Med Entomol 47, 43–52.

Aranda, C., Eritja, R., Roiz, D., 2006. First record and establishment of the mosquito *Aedes albopictus* in Spain. Med Vet Entomol 20, 150–152.

Aranda, C., Martínez, M.J., Montalvo, T., Eritja, R., Navero-Castillejos, J., Herreros, E., Marqués, E., Escosa, R., Corbella, I., Bigas, E., Picart, L., Jané, M., Barrabeig, I., Torner, N., Talavera, S., Vázquez, A., Sánchez-Seco, M.P., Busquets, N., 2018. Arbovirus surveillance: first dengue virus detection in local *Aedes albopictus* mosquitoes in Europe, Catalonia, Spain, 2015. Euro Surveill 23, 1700837.

Ayuntamiento de Vitoria-Gasteiz, 2021. Vitoria-Gasteiz obtiene el premio Global Green City Award. 2021. [WWW Document]. URL https://blogs.vitoria-gasteiz.org/medios/2019/09/06/vitoria-gasteiz-obtiene-el-premio-global-green-city-award/. (accessed 2.1.24).

Bahnck, C.M., Fonseca, D.M., 2006. Rapid Assay to Identify the two Genetic Forms of *Culex* (*Culex*) *pipiens* L. (Diptera: Culicidae) and Hybrid Populations. Am J Trop Med Hyg 75, 251–255.

Baldacchino, F., Arnoldi, D., Lapère, C., Rosà, R., Montarsi, F., Capelli, G., Rizzoli, A., 2017. Weak Larval Competition Between Two Invasive Mosquitoes *Aedes koreicus* and *Aedes albopictus* (Diptera: Culicidae). J Med Entomol 54, 1266–1272.

Balenghien, T., Fouque, F., Sabatier, P., Bicout, D.J., 2006. Horse-, Bird-, and Human-Seeking Behavior and Seasonal Abundance of Mosquitoes in a West Nile Virus Focus of Southern France. J Med Entomol 43, 936–946.

Balenghien, T., Vazeille, M., Grandadam, M., Schaffner, F., Zeller, H., Reiter, P., Sabatier, P., Fouque, F., Bicout, D.J., 2008. Vector Competence of Some French *Culex* and *Aedes* Mosquitoes for West Nile Virus. Vector-Borne Zoonotic Dis 8, 589–596.

Baneth, G., Thamsborg, S.M., Otranto, D., Guillot, J., Blaga, R., Deplazes, P., Solano-Gallego, L., 2016. Major Parasitic Zoonoses Associated with Dogs and Cats in Europe. J Comp Pathol 155, S54–S74.

Barceló, C., Blanda, V., del Castillo-Remiro, A., Chaskopoulou, A., Connelly, C.R., Ferrero-Gómez, L., La Russa, F., Parker-Crockett, C., Serafín-Pérez, I., Sousa, C.A., Torina, A., Vontas, J., Miranda, M.Á., 2022. Surveillance of invasive mosquito species in islands with focus on potential vectors of zoonotic diseases, in: Gutiérrez-López. Rafael, Logan. James G., Martínez-de la Puente. Josué (Eds.), Ecology of Diseases Transmitted by Mosquitoes to Wildlife. Brill | Wageningen Academic, pp. 179–207.

Barros, S.C., Ramos, F., Fagulha, T., Duarte, M., Henriques, A.M., Waap, H., Luís, T., Costa, T., Amador, R., Quintans, S., Fevereiro, M., 2017. West Nile virus in horses during the summer and autumn seasons of 2015 and 2016, Portugal. Vet Microbiol 212, 75–79.

Bartlett-Healy, K., Crans, W., Gaugler, R., 2008. Temporal and Spatial Synchrony of Culex territans (Diptera: Culicidae) with Their Amphibian Hosts. J Med Entomol 45, 1031–1038.

Bartlett-Healy, K., Unlu, I., Obenauer, P., Hughes, T., Healy, S., Crepeau, T., Farajollahi, A., Kesavaraju, B., Fonseca, D., Schoeler, G., Gaugler, R., Strickman, D., 2012. Larval Mosquito Habitat Utilization and Community Dynamics of *Aedes albopictus* and *Aedes japonicus* (Diptera: Culicidae). J Med Entomol 49, 813–824.

Barton, K., Barton, M.K., 2020. MuMIn: Multi-Model Inference. R package version 1.43.17. Version 1.

Batzer, D.P., 1993. Technique for surveying larval populations of *Coquillettidia perturbans*. J Am Mosq Control Assoc 9, 349–351.

Becker, N., Hoffmann, D., 2011. First record of *Culiseta longiareolata* (Macquart) for Germany. Eur Mosq Bull 29, 143–150.

Becker, N., Jöst, A., Weitzel, T., 2012. The *Culex pipiens* Complex in Europe. J Am Mosq Control Assoc 28, 53–67.

Becker, N., Petrić, D., Zgomba, M., Boase, C., Madon, M.B., Dahl, C., Kaiser, A., 2020. Mosquitoes, identification, ecology and control, 3rd ed. Springer International Publishing, Cham, Switzerland.

Beebe, N.W., 2018. DNA barcoding mosquitoes: advice for potential prospectors. Parasitology 145, 622–633.

Beier, J.C., Freier, J.E., 1984. Oral and Transovarial Transmission of la Crosse Virus by *Aedes atropalpus*. Am J Trop Med Hyg 33, 708–714.

Bertola, M., Mazzucato, M., Pombi, M., Montarsi, F., 2022. Updated occurrence and bionomics of potential malaria vectors in Europe: a systematic review (2000–2021). Parasit Vectors 15, 88.

Blagrove, M.S.C., Sherlock, K., Chapman, G.E., Impoinvil, D.E., McCall, P.J., Medlock, J.M., Lycett, G., Solomon, T., Baylis, M., 2016. Evaluation of the vector competence of a native UK mosquito *Ochlerotatus detritus* (*Aedes detritus*) for dengue, chikungunya and West Nile viruses. Parasit Vectors 9, 452.

Bødker, R., Klitgård, K., Byriel, D.B., Kristensen, B., 2014. Establishment of the West Nile virus vector, *Culex modestus*, in a residential area in Denmark. J Vector Ecol 39, 1–3.

Bonizzoni, M., Gasperi, G., Chen, X., James, A.A., 2013. The invasive mosquito species *Aedes albopictus*: current knowledge and future perspectives. Trends Parasitol 29, 460–468.

Bonnet, E., Fournet, F., Benmarhnia, T., Ouedraogo, S., Dabiré, R., Ridde, V., 2020. Impact of a community-based intervention on *Aedes aegypti* and its spatial distribution in Ouagadougou, Burkina Faso. Infect Dis Poverty 9, 61.

Börstler, J., Jöst, H., Garms, R., Krüger, A., Tannich, E., Becker, N., Schmidt-Chanasit, J., Lühken, R., 2016. Host-feeding patterns of mosquito species in Germany. Parasit Vectors 9, 318.

Borucki, M.K., Kempf, B.J., Blitvich, B.J., Blair, C.D., Beaty, B.J., 2002. La Crosse virus: replication in vertebrate and invertebrate hosts. Microbes Infect 4, 341–350.

Bosak, P.J., Crans, W.J., 2002. The structure and function of the larval siphon and spiracular apparatus of *Coquillettidia perturbans*. J Am Mosq Control Assoc 18, 280–283.

Boualam, M.A., Pradines, B., Drancourt, M., Barbieri, R., 2021. Malaria in Europe: A Historical Perspective. Front Med (Lausanne) 8, 691095.

Bravo-Barriga, D., de Almeida, A.P.G., Delacour-Estrella, S., Peña, R.E., Lucientes, J., Sánchez-Murillo, J.M., Frontera, E., 2021. Mosquito fauna in Extremadura (western Spain): Updated catalog with new records, distribution maps, and medical relevance. J Vector Ecol 46, 70–82.

Bravo-Barriga, D., Gomes, B., Almeida, A.P.G., Serrano-Aguilera, F.J., Pérez-Martín, J.E., Calero-Bernal, R., Reina, D., Frontera, E., Pinto, J., 2017. The mosquito fauna of the western region of Spain with emphasis on ecological factors and the characterization of *Culex pipiens* forms. J Vector Ecol 42, 136–147.

Bravo-Barriga, D., Parreira, R., Almeida, A.P.G., Calado, M., Blanco-Ciudad, J., Serrano-Aguilera, F.J., Pérez-Martín, J.E., Sánchez-Peinado, J., Pinto, J., Reina, D., Frontera, E., 2016. *Culex pipiens* as a potential vector for transmission of *Dirofilaria immitis* and other unclassified Filarioidea in Southwest Spain. Vet Parasitol 223, 173–180.

Bruce-Chwatt, L.J., De Zulueta, J., 1980. The rise and fall of malaria in Europe: a historico-epidemiological study. Published for the Regional Office for Europe of the World Health Organization by Oxford University Press, Walton Street, Oxford OX2 6DP.

Brugman, V., Hernández-Triana, L., Medlock, J., Fooks, A., Carpenter, S., Johnson, N., 2018. The Role of *Culex pipiens* L. (Diptera: Culicidae) in Virus Transmission in Europe. Int J Environ Res Public Health 15, 389.

Brugman, Victor A., England, M.E., Stoner, J., Tugwell, L., Harrup, L.E., Wilson, A.J., Medlock, J.M., Logan, J.G., Fooks, A.R., Mertens, P.P.C., Johnson, N., Carpenter, S., 2017. How often do mosquitoes bite humans in southern England? A standardised summer trial at four sites reveals spatial, temporal and site-related variation in biting rates. Parasit Vectors 10, 420.

Brugman, V. A., Hernández-Triana, L.M., England, M.E., Medlock, J.M., Mertens, P.P.C., Logan, J.G., Wilson, A.J., Fooks, A.R., Johnson, N., Carpenter, S., 2017. Blood-feeding patterns of native mosquitoes and insights into their potential role as pathogen vectors in the Thames estuary region of the United Kingdom. Parasit Vectors 10, 163.

Brugueras, S., Fernández-Martínez, B., Martínez-de la Puente, J., Figuerola, J., Porro, T.M., Rius, C., Larrauri, A., Gómez-Barroso, D., 2020. Environmental drivers, climate change and emergent diseases transmitted by mosquitoes and their vectors in southern Europe: A systematic review. Environ Res 191, 110038.

Brust, R.A., 1980. Disperal Behavior of Adult *Aedes sticticus* and *Aedes vexans* (Diptera: Culicidae) in Manitoba. Can Entomol 112, 31–42.

Bueno Marí, R., Jiménez Peydró, R., 2008. Malaria en España: aspectos entomológicos y perspectivas de futuro. Rev Esp Salud Publica 82, 467–479.

Bueno Marí, R., Jiménez-Peydró, R., 2011. Differences in mosquito (Diptera: Culicidae) biodiversity across varying climates and land-use categories in Eastern Spain. Entomol Fenn 22, 190–198.

Bueno-Marí, R., 2010. Tesis Doctoral: Bioecología, diversidad e interés epidemiológico de los culícidos mediterráneos (Diptera: Culicidae). Universitat de València.

Bueno-Marí, R., Bernués-Bañeres, A., Jiménez-Peydró, R., 2012. Updated checklist and distribution maps of mosquitoes (Diptera: Culicidae) of Spain. Eur Mosq Bull 30, 91–126.

Bueno-Marí, R., Jiménez-Peydró, R., 2012. Study of the malariogenic potential of Eastern Spain. Trop Biomed 29, 39–50.

Bueno-Marí, R., Jiménez-Peydró, R., 2011. *Anopheles plumbeus* Stephens, 1828: a neglected malaria vector in Europe. Malar Rep 1, e2.

Burkett-Cadena, N.D., 2013. Mosquitoes of the southeastern United States, Mosquitoes of the Southeastern United States. University of Alabama Press, Tuscaloosa, AL.

Burkett-Cadena, N.D., McClure, C.J.W., Estep, L.K., Eubanks, M.D., 2013. Hosts or habitats: What drives the spatial distribution of mosquitoes? Ecosphere 4, 1–16.

Burki, T., 2018. Increase of West Nile virus cases in Europe for 2018. Lancet 392, 1000.

Calba, C., Guerbois-Galla, M., Franke, F., Jeannin, C., Auzet-Caillaud, M., Grard, G., Pigaglio, L., Decoppet, A., Weicherding, J., Savaill, M.C., Munoz-Riviero, M., Chaud, P., Cadiou, B., Ramalli, L., Fournier, P., Noël, H., De Lamballerie, X., Paty, M.C., Leparc-Goffart, I., 2017. Preliminary report of an autochthonous chikungunya outbreak in France, July to September 2017. Euro Surveill 22, 5–10.

Calzolari, M., 2016. Mosquito-borne diseases in Europe: an emerging public health threat. Rep Parasitol 5, 1–12.

Calzolari, M., Bonilauri, P., Bellini, R., Albieri, A., Defilippo, F., Tamba, M., Tassinari, M., Gelati, A., Cordioli, P., Angelini, P., Dottori, M., 2013. Usutu Virus Persistence and West Nile Virus Inactivity in the Emilia-Romagna Region (Italy) in 2011. PLoS One 8, e63978.

Caminade, C., Kovats, S., Rocklov, J., Tompkins, A.M., Morse, A.P., Colón-González, F.J., Stenlund, H., Martens, P., Lloyd, S.J., 2014. Impact of climate change on global malaria distribution. Proc Natl Acad Sci 111, 3286–3291.

Camp, J. V., Bakonyi, T., Soltész, Z., Zechmeister, T., Nowotny, N., 2018. *Uranotaenia unguiculata* Edwards, 1913 are attracted to sound, feed on amphibians, and are infected with multiple viruses. Parasit Vectors 11, 456.

Campbell-Lendrum, D., Manga, L., Bagayoko, M., Sommerfeld, J., 2015. Climate change and vector-borne diseases: what are the implications for public health research and policy? Philos Trans R Soc Lond B Biol Sci 370, 20130552.

Campos, R.K., Rossi, S.L., Tesh, R.B., Weaver, S.C., 2023. Zoonotic mosquito-borne arboviruses: Spillover, spillback, and realistic mitigation strategies. Sci Transl Med 15, eadj2166.

Cancrini, G., Allende, E., Favia, G., Bornay, F., Antón, F., Simón, F., 2000. Canine dirofilariosis in two cities of southeastern Spain. Vet Parasitol 92, 81–86.

Cansado-Utrilla, C., Jeffries, C.L., Kristan, M., Brugman, V.A., Heard, P., Camara, G., Sylla, M., Beavogui, A.H., Messenger, L.A., Irish, S.R., Walker, T., 2020. An assessment of adult mosquito collection techniques for studying species abundance and diversity in Maferinyah, Guinea. Parasit Vectors 13, 150.

Capelli, G., Drago, A., Martini, S., Montarsi, F., Soppelsa, M., Delai, N., Ravagnan, S., Mazzon, L., Schaffner, F., Mathis, A., Di Luca, M., Romi, R., Russo, F., 2011. First report in Italy of the exotic mosquito species *Aedes* (Finlaya) *koreicus*, a potential vector of arboviruses and filariae. Parasit Vectors 4, 188.

Capelli, G., Genchi, C., Baneth, G., Bourdeau, P., Brianti, E., Cardoso, L., Danesi, P., Fuehrer, H.P., Giannelli, A., Ionică, A.M., Maia, C., Modrý, D., Montarsi, F., Krücken, J., Papadopoulos, E., Petrić, D., Pfeffer, M., Savić, S., Otranto, D., Poppert, S., Silaghi, C., 2018. Recent advances on *Dirofilaria repens* in dogs and humans in Europe. Parasit Vectors 11, 663.

Carlson, J., Keating, J., Mbogo, C.M., Kahindi, S., Beier, J.C., 2004. Ecological limitations on aquatic mosquito predator colonization in the urban environment. J Vector Ecol 29, 331–339.

Carpenter, S., Szmaragd, C., Barber, J., Labuschagne, K., Gubbins, S., Mellor, P., 2008. An assessment of *Culicoides* surveillance techniques in northern Europe: have we underestimated a potential bluetongue virus vector? J Appl Ecol 45, 1237–1245.

Cauchemez, S., Besnard, M., Bompard, P., Dub, T., Guillemette-Artur, P., Eyrolle-Guignot, D., Salje, H., Van Kerkhove, M.D., Abadie, V., Garel, C., Fontanet, A., Mallet, H.-P., 2016. Association between Zika virus and microcephaly in French Polynesia, 2013–15: a retrospective study. Lancet 387, 2125–2132.

CDC, 2018. Yellow Fever [WWW Document]. CDC. URL https://www.cdc.gov/yellow-fever/index.html (accessed 4.1.24).

Cebrián-Camisón, S., Martínez-de la Puente, J., Figuerola, J., 2020. A Literature Review of Host Feeding Patterns of Invasive *Aedes* Mosquitoes in Europe. Insects 11, 848.

Centro de Coordinación de Alertas y Emergencias Sanitarias, M. de S., 2023a. Identificación del mosquito *Aedes aegypti* en Santa Cruz de Tenerife [WWW Document]. URL https://www.sanidad.gob.es/areas/alertasEmergenciasSanitarias/alertasActuales/vectores/docs/20230206_Ae_aegypti_ERR.pdf (accessed 5.1.24).

Centro de Coordinación de Alertas y Emergencias Sanitarias, M. de S., 2023b. Evaluación Rápida de Riesgo: Agrupación de casos de dengue autóctono en Ibiza [WWW Document]. URL https://www.sanidad.gob.es/areas/alertasEmergenciasSanitarias/alertasActuales/dengue/docs/20230228_ERR_Dengue_autoctono.pdf (accessed 5.1.24).

Centro de Coordinación de Alertas y Emergencias Sanitarias, M. de S., 2022. Evaluación rápida de riesgo. Identificación del mosquito *Aedes aegypti* en la Isla de la Palma. [WWW Document]. URL https://www.sanidad.gob.es/areas/alertasEmergenciasSanitarias/alertasActuales/vectores/docs/20220504_Ae_aegypti_ERR.pdf (accessed 5.1.24).

Centro Nacional de Epidemiología. Instituto de Salud Carlos III., 2022. Informe epidemiológico sobre la situación de dengue en España. Años 2019, 2020 y 2021. [WWW Document]. URL https://www.isciii.es/QueHacemos/Servicios/VigilanciaSaludPublicaRENAVE/EnfermedadesTransmisibles/Documents/archivos%20A-Z/Dengue/INFORME_RENAVE_DENGUE%202019-2021.pdf (accessed 5.1.24).

Chaves, L.F., Friberg, M.D., Moji, K., 2020. Synchrony of globally invasive *Aedes* spp. immature mosquitoes along an urban altitudinal gradient in their native range. Sci Total Environ 734, 139365.

Chen, I.-C., Hill, J.K., Ohlemüller, R., Roy, D.B., Thomas, C.D., 2011. Rapid Range Shifts of Species Associated with High Levels of Climate Warming. Science 333, 1024–1026.

Chen, Y.-C., Wang, C.-Y., Teng, H.-J., Chen, C.-F., Chang, M.-C., Lu, L.-C., Lin, C., Jian, S.-W., Wu, H.-S., 2011. Comparison of the efficacy of CO2-baited and unbaited light traps, gravid traps, backpack aspirators, and sweep net collections for sampling mosquitoes infected with Japanese encephalitis virus. J Vector Ecol 36, 68–74.

Chevalier, V., Pépin, M., Plée, L., Lancelot, R., 2010. Rift Valley fever - a threat for Europe? Euro Surveill 15, 19506.

Chiesura, A., 2004. The role of urban parks for the sustainability of cities. Advances in Architecture Series 68, 129–138.

Chordá Olmos, F.A., 2014. Tesis Doctoral: Biología de mosquitos (Diptera: Culicidae) en enclaves representativos de la Comunidad Valenciana. Universitat de València, Valencia.

Chouin-Carneiro, T., David, M.R., de Bruycker Nogueira, F., dos Santos, F.B., Lourenço-de-Oliveira, R., 2020. Zika virus transmission by Brazilian *Aedes aegypti* and *Aedes albopictus* is virus dose and temperature-dependent. PLoS Negl Trop Dis 14, e0008527.

Chouin-Carneiro, T., Vega-Rua, A., Vazeille, M., Yebakima, A., Girod, R., Goindin, D., Dupont-Rouzeyrol, M., Lourenço-de-Oliveira, R., Failloux, A.-B., 2016. Differential Susceptibilities of *Aedes aegypti* and *Aedes albopictus* from the Americas to Zika Virus. PLoS Negl Trop Dis 10, e0004543.

Ciocchetta, S., Prow, N.A., Darbro, J.M., Frentiu, F.D., Savino, S., Montarsi, F., Capelli, G., Aaskov, J.G., Devine, G.J., 2018. The new European invader *Aedes* (*Finlaya*) *koreicus*: a potential vector of chikungunya virus. Pathog Glob Health 112, 107–114.

Ciota, A.T., 2017. West Nile virus and its vectors. Curr Opin Insect Sci 22, 28–36.

Cirujano, S., Soriano, O., Velasco, J.L., García-Valdecasas, A., Álvarez Cobelas, M., Moreno, M., 2003. Estudio de la Flora Acuática y la Fauna Bentónica y Nectónica del Parque Periférico de Salburua (Vitoria). Centro de Estudios Ambientales, Ingurugiro Galetarako Ikastegia. Vitoria-Gasteiz.

Clements, A.N., 2012. The biology of Mosquitoes. Vol 3: Transmission of Viruses and Interactions with Bacteria. CABI Publishing, Wallingford, United Kingdom.

Clements, A.N., 2000. The Biology of Mosquitoes. Vol 1: Development, Nutrition and Reproduction. CABI Publishing, Wallingford, United Kingdom.

Clements, A.N., 1999. The Biology of Mosquitoes. Vol 2: Sensory Reception and Behaviour. CABI Publishing, Wallingford, United Kingdom.

Cochet, A., Calba, C., Jourdain, F., Grard, G., Durand, G.A., Guinard, A., Noël, H., Paty, M.-C., Franke, F., 2022. Autochthonous dengue in mainland France, 2022: geographical extension and incidence increase. Euro Surveill 27, 2200818.

Collantes, F., Delacour, S., Alarcón-Elbal, P.M., Ruiz-Arrondo, I., Delgado, J.A., Torrell-Sorio, A., Bengoa, M., Eritja, R., Miranda, M.Á., Molina, R., Lucientes, J., 2015. Review of ten-years presence of *Aedes albopictus* in Spain 2004-2014: known distribution and public health concerns. Parasit Vectors 8, 1–11.

Collins, F.H., Paskewitz, S.M., 1996. A review of the use of ribosomal DNA (rDNA) to differentiate among cryptic *Anopheles* species. Insect Mol Biol 5, 1–9.

Condé, S., Richard, D., Leclère, A.-S., 2002. Europe's biodiversity - biogeographical regions and seas. European Environment Agency, Sweden.

Corbet, P.S., Smith, S.M., 1974. Diel periodicities of landing of nulliparous and parous *Aedes aegypti* (L.) at Dar es Salaam, Tanzania (Diptera, Culicidae). Bull Entomol Res 64, 111–121.

Crans, W.J., 2023. New Jersey Mosquito Species: *Culex territans* Walker [WWW Document]. Rutgers Center for Vector Biology. URL https://vectorbio.rutgers.edu/outreach/species/terr.htm (accessed 12.11.23).

Crocker, W., Maute, K., Webb, C., French, K., 2017. Mosquito assemblages associated with urban water bodies; implications for pest and public health threats. Landsc Urban Plan 162, 115–125.

Culverwell, C.L., Uusitalo, R.J., Korhonen, E.M., Vapalahti, O.P., Huhtamo, E., Harbach, R.E., 2021. The mosquitoes of Finland: updated distributions and bionomics. Med Vet Entomol 35, 1–29.

Cunze, S., Koch, L.K., Kochmann, J., Klimpel, S., 2016. *Aedes albopictus* and *Aedes japonicus* - two invasive mosquito species with different temperature niches in Europe. Parasit Vectors 573.

Dale, P.E.R., Knight, J.M., 2008. Wetlands and mosquitoes: a review. Wetl Ecol Manag 16, 255–276.

Dalla Pozza, G., Majori, G., 1992. First record of *Aedes albopictus* establishment in Italy. J Am Mosq Control Assoc 8, 318–320.

Danabalan, R., Monaghan, M.T., Ponsonby, D.J., Linton, Y.M., 2014. Occurrence and host preferences of *Anopheles maculipennis* group mosquitoes in England and Wales. Med Vet Entomol 28, 169–178.

Danielova, V., Holubova, J., 1977. Two more mosquito species proved as vectors of Tahyna virus in Czechoslovakia. Folia Parasitol (Praha) 24, 187–189.

Davis, J.K., Vincent, G., Hildreth, M.B., Kightlinger, L., Carlson, C., Wimberly, M.C., 2017. Integrating Environmental Monitoring and Mosquito Surveillance to Predict Vector-borne Disease: Prospective Forecasts of a West Nile Virus Outbreak. PLoS Curr Outbreaks 9.

Day, J.F., Edman, J.D., 1984. The importance of disease induced changes in mammalian body temperature to mosquito blood feeding. Comp Biochem Physiol A Physiol 77, 447–452.

Day, J.F., Shaman, J., 2008. Using hydrologic conditions to forecast the risk of focal and epidemic arboviral transmission in peninsular Florida. J Med Entomol 45, 458–465.

de Castro, A., 2004. Los artrópodos de la Sierra de Aralar. Sociedad de Ciencias Aranzadi, Donostia-San Sebastián.

De León, A., Arriba, A., de la Plaza, M.C., 1989. Caracterización Agroclimática del País Vasco, 1st ed. Ministerio de Agricultura, Pesca y Alimentación, Secretaría General Técnica, Centro de Publicaciones, D.L., Madrid, Spain, pp. 100–184.

Deblauwe, I., Ibáñez-Justicia, A., De Wolf, K., Smitz, N., Schneider, A., Stroo, A., Jacobs, F., Vanslembrouck, A., Gombeer, S., Dekoninck, W., Müller, R., Van Bortel, W., 2021. First Detections of *Culiseta longiareolata* (Diptera: Culicidae) in Belgium and the Netherlands. J Med Entomol 58, 2524–2532.

Dekoninck, W., Hendrickx, F., Bortel, W. Van, Versteirt, V., Coosemans, M., Damiens, D., Hance, T., De Clercq, E.M., Hendrickx, G., Schaffner, F., Grootaert, P., 2011. Human-Induced Expanded Distribution of *Anopheles plumbeus*, Experimental Vector of West Nile Virus and a Potential Vector of Human Malaria in Belgium. J Med Entomol 48, 924–928.

Delacour, S., Barandika, J.F., García-Pérez, A.L., Collantes, F., Ruiz-Arrondo, I., Alarcón-Elbal, P.M., Bengoa, M., Delgado, J.A., Juste, R.A., Molina, R., Lucientes, J., 2015. Detección temprana de mosquito tigre, *Aedes albopictus* (Skuse, 1894), en el País Vasco (España). An Biol 37, 25–30.

Delgado-Serra, S., Viader, M., Ruiz-Arrondo, I., Miranda, M.Á., Barceló, C., Bueno-Marí, R., Hernández-Triana, L.M., Miquel, M., Lester, K., Jurado-Rivera, J.A., Paredes-Esquivel, C., 2021. Molecular Characterization of Mosquito Diversity in the Balearic Islands. J Med Entomol 58, 608–615.

Delisle, E., Rousseau, C., Broche, B., Leparc-Goffart, I., L'ambert, G., Cochet, A., Prat, C., Foulongne, V., Ferré, J.B., Catelinois, O., Flusin, O., Tchernonog, E., Moussion, I.E., Wiegandt, A., Septfons, A., Mendy, A., Moyano, M.B., Laporte, L., Maurel, J., Jourdain, F., Reynes, J., Paty, M.C., Golliot, F., 2015. Chikungunya outbreak in Montpellier, France, september to october 2014. Euro Surveill 20, 21108.

Dennett, J.A., Vessey, N.Y., Parsons, R.E., 2004. A comparison of seven traps used for collection of *Aedes albopictus* and *Aedes aegypti* originating from a large tire repository in Harris County (Houston), Texas. J Am Mosq Control Assoc 20, 342–349.

Detinova, T.S., 1962. Age-Grouping Methods in Diptera of Medical Importance: With Special Reference to Some Vectors of Malaria. J Parasitol 48, 456.

Detinova, T.S., Smelova, V.A., 1973. On the medical importance of mosquitoes (Culicidae, Diptera) of the fauna of the Soviet Union. Meditsinskaya Parazitologiya i Parazitarnye Bolezni 42, 455–471.

Drouin, A., Chevalier, V., Durand, B., Balenghien, T., 2022. Vector Competence of Mediterranean Mosquitoes for Rift Valley Fever Virus: A Meta-Analysis. Pathogens 11, 503.

ECDC, 2023a. *Aedes triseriatus* - Factsheet for experts [WWW Document]. European Centre for Disease Prevention and Control. URL https://www.ecdc.europa.eu/en/disease-vectors/facts/mosquito-factsheets/aedes-triseriatus (accessed 5.1.24).

ECDC, 2023b. 'Reverse' identification key for mosquito species [WWW Document]. European Centre for Disease Prevention and Control. URL https://www.ecdc.europa.eu/en/infectious-disease-topics/disease-vectors/reverse-identification-key-mosquito-species (accessed 5.1.24).

ECDC, 2023c. Distribution maps of invasive mosquitoes [WWW Document]. European Centre for Disease Prevention and Control. URL https://www.ecdc.europa.eu/en/disease-vectors/surveillance-and-disease-data/mosquito-maps (accessed 5.1.24).

ECDC, 2018. Field sampling methods for mosquitoes, sandflies, biting midges and ticks. European Centre for Disease Prevention and Control, Stockholm.

ECDC, 2014. Guidelines for the Surveillance of Native Mosquitoes in Europe. European Centre for Disease Prevention and Control, Stockholm.

ECDC, 2012. Guidelines for the Surveillance of Invasive Mosquitoes in Europe. European Centre for Disease Prevention and Control, Stockholm.

ECDC, EFSA, 2018. The importance of vector abundance and seasonality, European Centre for Disease Prevention and Control and European Food Safety Authority. Stockholm and Parma.

Edman, J.D., Kale, H.W., 1971. Host Behavior: Its Influence on the Feeding Success of Mosquitoes. Ann Entomol Soc Am 64, 513–516.

Epstein, P.R., 2005. Climate Change and Human Health. New Engl J Med 353, 1433–1436.

Eritja, R., Delacour-Estrella, S., Ruiz-Arrondo, I., González, M.A., Barceló, C., García-Pérez, A.L., Lucientes, J., Miranda, M.Á., Bartumeus, F., 2021. At the tip of an iceberg: citizen science and active surveillance collaborating to broaden the known distribution of *Aedes japonicus* in Spain. Parasit Vectors 14, 375.

Eritja, R., Escosa, R., Lucientes, J., Marquès, E., Roiz, D., Ruiz, S., 2005. Worldwide invasion of vector mosquitoes: present European distribution and challenges for Spain. Biol Invasions 7, 87–97.

Eritja, R., Palmer, J.R.B., Roiz, D., Sanpera-Calbet, I., Bartumeus, F., 2017. Direct Evidence of Adult *Aedes albopictus* Dispersal by Car. Sci Rep 7, 14399.

Eritja, R., Ruiz-Arrondo, I., Delacour-Estrella, S., Schaffner, F., Álvarez-Chachero, J., Bengoa, M., Puig, M.Á., Melero-Alcíbar, R., Oltra, A., Bartumeus, F., 2019. First detection of *Aedes japonicus* in Spain: An unexpected finding triggered by citizen science. Parasit Vectors 12, 53.

Erraguntla, M., Dave, D., Zapletal, J., Myles, K., Adelman, Z.N., Pohlenz, T.D., Lawley, M., 2021. Predictive model for microclimatic temperature and its use in mosquito population modeling. Sci Rep 11, 18909.

Estep, L.K., McClure, C.J.W., Burkett-Cadena, N.D., Hassan, H.K., Unnasch, T.R., Hill, G.E., 2012. Developing Models for the Forage Ratios of *Culiseta melanura* and *Culex erraticus* Using Species Characteristics for Avian Hosts. J Med Entomol 49, 378–387.

Estrada-Franco, J.G., Fernández-Santos, N.A., Adebiyi, A.A., López-López, M. de J., Aguilar-Durán, J.A., Hernández-Triana, L.M., Prosser, S.W.J., Hebert, P.D.N., Fooks, A.R., Hamer, G.L., Xue, L., Rodríguez-Pérez, M.A., 2020. Vertebrate-*Aedes aegypti* and *Culex quinquefasciatus* (Diptera)-arbovirus transmission networks: Non-human feeding revealed by meta-barcoding and next-generation sequencing. PLoS Negl Trop Dis 14, e0008867.

Eubanks, M.D., Hassan, H.K., Unnasch, T.R., Guyer, C., Graham, S.P., Burkett-Cadena, N.D., Katholi, C.R., 2008. Blood Feeding Patterns of Potential Arbovirus Vectors of the Genus *Culex* Targeting Ectothermic Hosts. Am J Trop Med Hyg 79, 809–815.

Euskalmet, 2022. El clima en Euskadi [WWW Document]. URL https://www.euskalmet.euskadi.eus/clima/euskadi/ (accessed 5.1.24).

EUSTAT, 2022. Población de la C.A. de Euskadi por ámbitos territoriales, según sexo y densidad de población. 01/01/2022 [WWW Document]. Euskal Estatistika Erakundea-Instituto Vasco de Estadística. URL https://www.eustat.eus/elementos/ele0011400/Poblacion_de_la_CA_de_Euskadi_por_ambitos_territoriales_segun_sexo_y_densidad_de_poblacion/tbl0011429_c.html (accessed 5.1.24).

EUSTAT, 2021. Estructura general y orgánica, y calificación del suelo de la C.A. de Euskadi por ámbitos territoriales (Ha). 2021 [WWW Document]. Euskal Estatistika Erakundea Instituto Vasco de Estadística. URL https://www.eustat.eus/elementos/ele0005400/estructura-general-y-organica-y-calificacion-del-suelo-de-la-ca-de-euskadi-por-ambitos-territoriales-ha/tbl0005454_c.html (accessed 5.1.24).

EUSTAT, 2020. EUSTAT—Instituto Vasco de Estadística [WWW Document]. URL https://en.eustat.eus/estadisticas/tema_351/opt_0/temas.html (accessed 5.1.24).

Ewing, D.A., Cobbold, C.A., Purse, B.V., Nunn, M.A., White, S.M., 2016. Modelling the effect of temperature on the seasonal population dynamics of temperate mosquitoes. J Theor Biol 400, 65–79.

Ewing, D.A., Purse, B. V., Cobbold, C.A., Schäfer, S.M., White, S.M., 2019. Uncovering mechanisms behind mosquito seasonality by integrating mathematical models and daily empirical population data: *Culex pipiens* in the UK. Parasit Vectors 12, 74.

Farooq, Z., Sjödin, H., Semenza, J.C., Tozan, Y., Sewe, M.O., Wallin, J., Rocklöv, J., 2023. European projections of West Nile virus transmission under climate change scenarios. One Health 16, 100509.

Ferraccioli, F., Riccetti, N., Fasano, A., Mourelatos, S., Kioutsioukis, I., Stilianakis, N.I., 2023. Effects of climatic and environmental factors on mosquito population inferred from West Nile virus surveillance in Greece. Sci Rep 13, 18803.

Ferraguti, M., Heesterbeek, H., Martínez-de la Puente, J., Jiménez-Clavero, M.Á., Vázquez, A., Ruiz, S., Llorente, F., Roiz, D., Vernooij, H., Soriguer, R., Figuerola, J., 2021. The role of different *Culex* mosquito species in the transmission of West Nile virus and avian malaria parasites in Mediterranean areas. Transbound Emerg Dis 68, 920–930.

Ferraguti, M., Magallanes, S., Ibánez-Justicia, A., 2022. Implication of human landscape transformation on mosquito populations, in: Ecology and Control of Vector-Borne Diseases. Wageningen Academic Publishers, pp. 279–283.

Ferraguti, M., Martínez-de la Puente, J., Brugueras, S., Millet, J.P., Rius, C., Valsecchi, A., Figuerola, J., Montalvo, T., 2023. Spatial distribution and temporal dynamics of invasive and native mosquitoes in a large Mediterranean city. Sci Total Environ 896, 165322.

Ferraguti, M., Martínez-de la Puente, J., Roiz, D., Ruiz, S., Soriguer, R., Figuerola, J., 2016. Effects of landscape anthropization on mosquito community composition and abundance. Sci Rep 6, 29002.

Figuerola, J., Jiménez-Clavero, M.Á., Ruíz-López, M.J., Llorente, F., Ruiz, S., Hoefer, A., Aguilera-Sepúlveda, P., Jiménez-Peñuela, J., García-Ruiz, O., Herrero, L., Soriguer, R.C., Fernández Delgado, R., Sánchez-Seco, M.P., Martínez-de la Puente, J., Vázquez, A., 2022. A One Health view of the West Nile virus outbreak in Andalusia (Spain) in 2020. Emerg Microbes Infect 11, 2570–2578.

Figuerola, J., Muñoz, J., Soriguer, R., Roussel, M., Roiz, D., Ruiz, S., 2012. Efficacy of Mosquito Traps for Collecting Potential West Nile Mosquito Vectors in a Natural Mediterranean Wetland. Am J Trop Med Hyg 86, 642–648.

Fikrig, K., Harrington, L.C., 2021. Understanding and interpreting mosquito blood feeding studies: the case of *Aedes albopictus*. Trends Parasitol 37, 959–975.

Fischer, S., Schweigmann, N., 2004. *Culex* mosquitoes in temporary urban rain pools: seasonal dynamics and relation to environmental variables. J Vector Ecol 29, 18803.

Foley, D.H., Rueda, L.M., Wilkerson, R.C., 2007. Insight into global mosquito biogeography from country species records. J Med Entomol 44, 554–567.

Fonseca, D.M., Keyghobadi, N., Malcolm, C.A., Mehmet, C., Schaffner, F., Mogi, M., Fleischer, R.C., Wilkerson, R.C., 2004. Emerging Vectors in the *Culex pipiens* Complex. Science 303, 1535–1538.

Foster, W.A., Walker, E.D., 2019. Mosquitoes (Culicidae), in: Medical and Veterinary Entomology. Academic Press, pp. 261–325.

Fouet, C., Kamdem, C., 2019. Integrated Mosquito Management: Is Precision Control a Luxury or Necessity? Trends Parasitol 35, 85–95.

Franklinos, L.H. V, Jones, K.E., Redding, D.W., Abubakar, I., 2019. The effect of global change on mosquito-borne disease. Lancet Infect Dis 19, e302–e312.

Frías-Saéz, J., 2022. Salburua un año en la vida de los humedales. Ediciones Beta III Milenio, Bilbao.

Früh, L., Kampen, H., Koban, M.B., Pernat, N., Schaub, G.A., Werner, D., 2020. Oviposition of *Aedes japonicus japonicus* (Diptera: Culicidae) and associated native species in relation to season, temperature and land use in western Germany. Parasit Vectors 13, 623.

Fundación Affinity, 2018. Resultados del estudio del abandono en España 2018 [WWW Document]. URL https://www.fundacion-affinity.org/blog/resultados-del-estudio-del-abandono-en-españa-2018 (accessed 4.1.24).

Fyie, L.R., Gardiner, M.M., Meuti, M.E., 2021. Artificial light at night alters the seasonal responses of biting mosquitoes. J Insect Physiol 129, 104194.

Gad, A.M., Farid, H.A., Ramzy, R.R.M., Riad, M.B., Presley, S.M., Cope, S.E., Hassan, M.M., Hassan, A.N., 1999. Host Feeding of Mosquitoes (Diptera: Culicidae) Associated with the Recurrence of Rift Valley Fever in Egypt. J Med Entomol 36, 709–714.

Ganassi, S., De Cristofaro, A., Di Criscio, D., Petrarca, S., Leopardi, C., Guarnieri, A., Pietrangelo, L., Venditti, N., Di Marco, R., Petronio Petronio, G., 2022. The new invasive mosquito species *Aedes koreicus* as vector-borne diseases in the European area, a focus on Italian region: What we know from the scientific literature. Front Microbiol 13, 931994.

Gangoso, L., Aragonés, D., Martínez-de la Puente, J., Lucientes, J., Delacour-Estrella, S., Estrada Peña, R., Montalvo, T., Bueno-Marí, R., Bravo-Barriga, D., Frontera, E., Marqués, E., Ruiz-Arrondo, I., Muñoz, A., Oteo, J.A., Miranda, M.A., Barceló, C., Arias Vázquez, M.S., Silva-Torres, M.I., Ferraguti, M., Magallanes, S., Muriel, J., Marzal, A., Aranda, C., Ruiz, S., González, M.A., Morchón, R., Gómez-Barroso, D., Figuerola, J., 2020. Determinants of the current and future distribution of the West Nile virus mosquito vector *Culex pipiens* in Spain. Environ Res 188, 109837.

García San Miguel Rodríguez-Alarcón, L., Fernández-Martínez, B., Sierra Moros, M.J., Vázquez, A., Julián Pachés, P., García Villacieros, E., Gómez Martín, M.B., Figuerola Borras, J., Lorusso, N., Ramos Aceitero, J.M., Moro, E., de Celis, A., Oyonarte, S., Mahillo, B., Romero González, L.J., Sánchez-Seco, M.P., Suárez Rodríguez, B., Ameyugo Catalán, U., Ruiz Contreras, S., Pérez-Olmeda, M., Simón Soria, Fernando, 2021. Unprecedented increase of West Nile virus neuroinvasive disease, Spain, summer 2020. Euro Surveill 26, 2002010.

García-Bocanegra, I., Belkhiria, J., Napp, S., Cano-Terriza, D., Jiménez-Ruiz, S., Martínez-López, B., 2018. Epidemiology and spatio-temporal analysis of West Nile Virus in horses in Spain between 2010 and 2016. Transbound Emerg Dis 65, 567–577.

García-Bocanegra, I., Jaén-Téllez, J.A., Napp, S., Arenas-Montes, A., Fernández-Morente, M., Fernández-Molera, V., Arenas, A., 2011. West Nile Fever Outbreak in Horses and Humans, Spain, 2010. Emerg Infect Dis 17, 2397–2399.

Garrido, M., Veiga, J., Garrigós, M., Morales-Yuste, M., Recuero-Gil, J., Martínez-de la Puente, J., 2024. *Aedes albopictus* in a recently invaded area in Spain: effects of trap type, locality, and season on mosquito captures. Sci Rep 14, 2131.

Gatt, P., 2009. *Culex (Culex) perexiguus* Theobald (Diptera: Culicidae), a mosquito species new to the Maltese fauna. Eur Mosq Bull 27, 7–9.

Gibson, G., Torr, S.J., 1999. Visual and olfactory responses of haematophagous Diptera to host stimuli. Med Vet Entomol 13, 2–23.

Gillett, J.D., 1955. Variation in the Hatching-response of *Aedes* Eggs (Diptera: Culicidae). Bull Entomol Res 46, 241–254.

Gillies, M.T., 1980. The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a review. Bull Entomol Res 70, 525–532.

Gilotra, S.K., Rozeboom, L.E., Bhattacharya, N.C., 1967. Observations on possible competitive displacement between populations of *Aedes aegypti* Linnaeus and *Aedes albopictus* Skuse in Calcutta. Bull World Health Organ 37, 437–446.

Giménez, N., Barahona, M., Casasa, A., Domingo, A., Gavagnach, M., Martí, C., 2007. Llegada de *Aedes albopictus* a España, un nuevo reto para la salud pública. Gac Sanit 21, 25–28.

Giunti, G., Becker, N., Benelli, G., 2023a. Invasive mosquito vectors in Europe: From bioecology to surveillance and management. Acta Trop 239, 106832.

Giunti, G., Wilke, A.B.B., Beier, J.C., Benelli, G., 2023b. What Do We Know About the Invasive Mosquitoes *Aedes atropalpus* and *Aedes triseriatus*? Curr Trop Med Rep 10, 41–46.

Gjenero-Margan, I., Aleraj, B., Krajcar, D., Lesnikar, V., Klobučar, A., Pem-Novosel, I., Kurečić-Filipović, S., Komparak, S., Martić, R., Đuričić, S., Betica-Radić, L., Okmadžić, J., Vilibić-Čavlek, T., Babić-Erceg, A., Turković, B., Avšić-Županc, T., Radić, I., Ljubić, M., Šarac, K., Benić, N., Mlinarić-Galinović, G., 2011. Autochthonous dengue fever in Croatia, August–September 2010. Euro Surveill 16, 19805.

Gjullin, C.M., Yates, W.W., Stage, H.H., 1950. Studies on *Aedes Vexans* (Meig.) and *Aedes Sticticus* (Meig.), Flood-Water Mosquitoes, in the Lower Columbia River Valley. Ann Entomol Soc Am 43, 262–275.

Goiri, F., González, M.A., Goikolea, J., Oribe, M., de Castro, V., Delacour, S., Lucientes, J., Ortega-Araiztegi, I., Barandika, J.F., García-Pérez, A.L., 2020. Progressive Invasion of *Aedes albopictus* in Northern Spain in The Period 2013–2018 and A Possible Association with The Increase in Insect Bites. Int J Environ Res Public Health 17, 1678.

Goldberg, T.L., Anderson, T.K., Hamer, G.L., 2010. West Nile virus may have hitched a ride across the Western United States on *Culex tarsalis* mosquitoes. Mol Ecol 19, 1518–1519.

Golding, N., Nunn, M.A., Medlock, J.M., Purse, B. V, Vaux, A.G., Schäfer, S.M., 2012. West Nile virus vector *Culex modestus* established in southern England. Parasit Vectors 5, 32.

Gomes, B., Sousa, C.A., Vicente, J.L., Pinho, L., Calderón, I., Arez, E., Almeida, A.P., Donnelly, M.J., Pinto, J., 2013. Feeding patterns of molestus and pipiens forms of *Culex pipiens* (Diptera: Culicidae) in a region of high hybridization. Parasit Vectors 6, 93.

Gómez, M. V., 1998. Reflective images: the case of urban regeneration in Glasgow and Bilbao. Int J Urban Reg Res 22, 106–121.

González, M., Alarcón-Elbal, P.M., Valle-Mora, J., Goldarazena, A., 2016. Comparison of different light sources for trapping Culicoides biting midges, mosquitoes and other dipterans. Vet Parasitol 226, 44–49.

González, M.A., 2022. Insectos de Importancia Médico-Veterinaria en el Norte de España. Self-edited, Gasteiz.

González, M.A., Cevidanes, A., Goiri, F., Barandika, J.F., García-Pérez, A.L., 2021. Diversity and distribution of larval habitats of mosquitoes (Diptera: Culicidae) in northern Spain: from urban to natural areas. J Vector Ecol 46, 173–185.

González, M.A., Delacour-Estrella, S., Bengoa, M., Barceló, C., Bueno-Marí, R., Eritja, R., Ruiz-Arrondo, I., 2022. A Survey on Native and Invasive Mosquitoes and Other Biting Dipterans in Northern Spain. Acta Parasitol 67, 867–877.

González, M.A., López, S., Alarcón-Elbal, P.M., 2015. Blood-feeding Diptera (Culicidae and Ceratopogonidae) in an urban park of the city of Vitoria-Gasteiz (Basque Country, Spain). J Eur Mosq Control Assoc 33, 10–14.

González, M.A., Prosser, S.W., Hernández-Triana, L.M., Alarcón-Elbal, P.M., Goiri, F., López, S., Ruiz-Arrondo, I., Hebert, P.D.N., García-Pérez, A.L., 2020. Avian Feeding Preferences of *Culex pipiens* and *Culiseta* spp. Along an Urban-to-Wild Gradient in Northern Spain. Front Ecol Evol 8, 568835.

González, Mikel Alexander, Rodríguez Sosa, M.A., Vásquez Bautista, Y.E., Diéguez Fernández, L., Borge de Prada, M., Guerrero, K.A., Alarcón-Elbal, P.M., González, Mikel A., Rodríguez Sosa, M.A., Vásquez Bautista, Y.E., Diéguez Fernández, L., Borge de Prada, M., Guerrero, K.A., Alarcón-Elbal, P.M., 2019. Micro-environmental features associated to container-dwelling mosquitoes (Diptera: Culicidae) in an urban cemetery of the Dominican Republic. Rev Biol Trop 67, 132–145.

Gossner, C.M., Fournet, N., Frank, C., Fernández-Martínez, B., Del Manso, M., Gomes Dias, J., de Valk, H., 2022. Dengue virus infections among European travellers, 2015 to 2019. Euro Surveill 27, 2001937.

Gossner, C.M., Marrama, L., Carson, M., Allerberger, F., Calistri, P., Dilaveris, D., Lecollinet, S., Morgan, D., Nowotny, N., Paty, M., Pervanidou, D., Rizzo, C., Roberts, H., Schmoll, F., Van Bortel, W., Gervelmeyer, A., 2017. West Nile Virus surveillance in europe: Moving towards an integrated animal-human-vector approach. Euro Surveill 22, 30526.

Gottdenker, N.L., Streicker, D.G., Faust, C.L., Carroll, C.R., 2014. Anthropogenic Land Use Change and Infectious Diseases: A Review of the Evidence. Ecohealth 11, 619–632.

Gouveia de Almeida, A.P., 2011. Mosquitoes (Diptera, Culicidae) and their medical importance for Portugal: challenges for the 21st century. [In Portuguese]. Acta Med Port 24, 961–74.

Grandadam, M., Caro, V., Plumet, S., Thiberge, J.-M., Souarès, Y., Failloux, A.-B., Tolou, H.J., Budelot, M., Cosserat, D., Leparc-Goffart, I., Desprès, P., 2011. Chikungunya Virus, Southeastern France. Emerg Infect Dis 17, 910–913.

Grego, B., Zamburlini, R., 2021. New Records of *Aedes (Rusticoidus) rusticus* (Rossi, 1790) (Diptera, Culicidae) From Friuli Venezia Giulia Region (Northern Italy). Gortania Botanica Zoologia 43, 95–98.

Guerenstein, P.G., Hildebrand, J.G., 2008. Roles and Effects of Environmental Carbon Dioxide in Insect Life. Annu Rev Entomol 53, 161–178.

Gunay, F., Picard, M., Robert, V., 2020. MosKeyTool, an interactive identification key for mosquitoes of Euro-Mediterranean. Version 2.2.

Guo, F., Bonebrake, T.C., Gibson, L., 2019. Land-Use Change Alters Host and Vector Communities and May Elevate Disease Risk. Ecohealth 16, 647–658.

Gutiérrez-López, R., la Puente, J.M., Borras, J.F., 2022. 6. Tools for mosquito blood meal identification, in: Ecology of Diseases Transmitted by Mosquitoes to Wildlife. Brill | Wageningen Academic, pp. 113–123.

Haba, Y., McBride, L., 2022. Origin and status of *Culex pipiens* mosquito ecotypes. Curr Biol 32, R237–R246.

Hagan, R.W., Didion, E.M., Rosselot, A.E., Holmes, C.J., Siler, S.C., Rosendale, A.J., Hendershot, J.M., Elliot, K.S.B., Jennings, E.C., Nine, G.A., Perez, P.L., Rizlallah, A.E., Watanabe, M., Romick-Rosendale, L.E., Xiao, Y., Rasgon, J.L., Benoit, J.B., 2018. Dehydration prompts increased activity and blood feeding by mosquitoes. Sci Rep 8, 6804.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor. Nucleic Acids Symp Ser 41, 95–98.

Harbach, R.E., 2023. Valid Species | Mosquito Taxonomic Inventory [WWW Document]. URL https://mosquito-taxonomic-inventory.myspecies.info/valid-species-list

Harbach, R.E., 2007. The Culicidae (Diptera): A review of taxonomy, classification and phylogeny. Zootaxa 591–638.

Harbach, R.E., 1999. The identity of *Culex perexiguus* versus *Culex univittatus* in southern Europe. J Eur Mosq Control Assoc 4, 7.

Harbach, R.E., 1988. The mosquitoes of the subgenus *Culex* in southwestern Asia and Egypt (Diptera: Culicidae). Contrib Am Entomol Inst 24.

Harbach, R.E., Dallimore, T., Briscoe, A.G., Culverwell, C.L., Vaux, A.G.C., Medlock, J.M., 2017. *Aedes nigrinus* (Eckstein, 1918) (Diptera, Culicidae), a new country record for England, contrasted with *Aedes sticticus* (Meigen, 1838). Zookeys 671, 119–130.

Hartman, A., 2017. Rift valley fever. Clin Lab Med 37, 285–301.

Hawkes, F.M., Medlock, J.M., Vaux, A.G.C., Cheke, R.A., Gibson, G., 2020. Wetland Mosquito Survey Handbook: Assessing the suitability of British wetlands for mosquitoes. Natural Resources Institute, Chatham, UK.

Hebert, P.D.N., Cywinska, A., Ball, S.L., deWaard, J.R., 2003. Biological identifications through DNA barcodes. Proc R Soc Lond B Biol Sci 270, 313–321.

Hernández, R., Caño, A., 1999. Urdaibai. Bilbao Bizkaia Kutxa, Bilbao.

Hernandez-Colina, A., Gonzalez-Olvera, M., Lomax, E., Townsend, F., Maddox, A., Hesson, J.C., Sherlock, K., Ward, D., Eckley, L., Vercoe, M., Lopez, J., Baylis, M., 2021. Blood-feeding ecology of mosquitoes in two zoological gardens in the United Kingdom. Parasit Vectors 14, 249.

Hernández-Triana, L.M., Brugman, V.A., Nikolova, N.I., Ruiz-Arrondo, I., Barrero, E., Thorne, L., de Marco, M.F., Krüger, A., Lumley, S., Johnson, N., Fooks, A.R., 2019. DNA barcoding of British mosquitoes (Diptera, Culicidae) to support species identification, discovery of cryptic genetic diversity and monitoring invasive species. Zookeys 832, 57–76.

Hernández-Triana, L.M., Brugman, V.A., Pramual, P., Barrero, E., Nikolova, N.I., Ruiz-Arrondo, I., Kaiser, A., Krüger, A., Lumley, S., Osório, H.C., Ignjatović-Ćupina, A., Petrić, D., Laure Setier-Rio, M., Bødker, R., Johnson, N., 2020. Genetic diversity and population structure of *Culex modestus* across Europe: does recent appearance in the United Kingdom reveal a tendency for geographical spread? Med Vet Entomol 34, 86–96.

Hernández-Triana, L.M., Brugman, V.A., Prosser, S.W.J., Weland, C., Nikolova, N., Thorne, L., Marco, M.F., Fooks, A.R., Johnson, N., 2017. Molecular approaches for blood meal analysis and species identification of mosquitoes (Insecta: Diptera: Culicidae) in rural locations in southern England, United Kingdom. Zootaxa 4250.

Hernández-Triana, L.M., Prosser, S.W., Rodríguez-Perez, M.A., Chaverri, L.G., Hebert, P.D.N., Ryan Gregory, T., 2014. Recovery of DNA barcodes from blackfly museum specimens (Diptera: Simuliidae) using primer sets that target a variety of sequence lengths. Mol Ecol Resour 14, 508–518.

Hesson, J.C., Rettich, F., Merdic, E., Vignjevic, G., Östman, Ö., Schäfer, M., Schaffner, F., Foussadier, R., Besnard, G., Medlock, J., Scholte, E. -J., Lundström, J.O., 2014. The arbovirus vector *Culex torrentium* is more prevalent than *Culex pipiens* in northern and central Europe. Med Vet Entomol 28, 179–186.

Heym, E.C., Kampen, H., Fahle, M., Hohenbrink, T.L., Schäfer, M., Scheuch, D.E., Walther, D., 2017. *Anopheles plumbeus* (Diptera: Culicidae) in Germany: updated geographic distribution and public health impact of a nuisance and vector mosquito. Trop Med Int Heal 22, 103–112.

Hobbs, J.H., Hughes, E.A., Eichold, B.H., 1991. Replacement of *Aedes aegypti* by *Aedes albopictus* in Mobile, Alabama. J Am Mosq Control Assoc 7, 488–489.

Hohmeister, N., Werner, D., Kampen, H., 2021. The invasive Korean bush mosquito *Aedes koreicus* (Diptera: Culicidae) in Germany as of 2020. Parasit Vectors 14, 575.

Hoick, A.R., Meek, C.L., 1991. Comparison of Sampling Techniques for Adult Mosquitoes and Other Nematocera in Open Vegetation. J Entomol Sci 26, 231–236.

Holderman, C.J., Gezan, S.A., Stone, A.E.S., Connelly, C.R., Kaufman, P.E., 2018. Mosquitoes (Diptera: Culicidae) Collected from Residential Yards and Dog Kennels in Florida Using Two Aspirators, a Sweep Net, or a CDC Trap. J Med Entomol 55, 230–236.

Huang, S., Hamer, G.L., Molaei, G., Walker, E.D., Goldberg, T.L., Kitron, U.D., Andreadis, T.G., 2009. Genetic Variation Associated with Mammalian Feeding in *Culex pipiens* from a West Nile Virus Epidemic Region in Chicago, Illinois. Vector-Borne Zoonotic Dis 9, 637–642.

Hubálek, Z., Halouzka, J., 1999. West Nile Fever–a Reemerging Mosquito-Borne Viral Disease in Europe. Emerg Infect Dis 5, 643–650.

Huber, K., Jansen, S., Leggewie, M., Badusche, M., Schmidt-Chanasit, J., Becker, N., Tannich, E., Becker, S.C., 2014. *Aedes japonicus japonicus* (Diptera: Culicidae) from Germany have vector competence for Japan encephalitis virus but are refractory to infection with West Nile virus. Parasitol Res 113, 3195–3199.

Hulme, P.E., 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. J Appl Ecol 46, 10–18.

Ibáñez-Justicia, A., 2020. Pathways for introduction and dispersal of invasive *Aedes* mosquito species in Europe: a review. J Eur Mosq Control Assoc 38, 1–10.

Ibañez-Justicia, A., Cianci, D., 2015. Modelling the spatial distribution of the nuisance mosquito species *Anopheles plumbeus* (Diptera: Culicidae) in the Netherlands. Parasit Vectors 8, 258.

Ibañez-Justicia, A., Gloria-Soria, A., den Hartog, W., Dik, M., Jacobs, F., Stroo, A., 2017. The first detected airline introductions of yellow fever mosquitoes (*Aedes aegypti*) to Europe, at Schiphol International airport, the Netherlands. Parasit Vectors 10, 603.

Ibáñez-Justicia, A., Smitz, N., den Hartog, W., van de Vossenberg, B., De Wolf, K., Deblauwe, I., Van Bortel, W., Jacobs, F., Vaux, A.G.C., Medlock, J.M., Stroo, A., 2020. Detection of Exotic Mosquito Species (Diptera: Culicidae) at International Airports in Europe. Int J Environ Res Public Health 17, 3450.

Ibanez-Justicia, A., Stroo, A., Dik, M., Beeuwkes, J., Scholte, E.J., 2015. National Mosquito (Diptera: Culicidae) Survey in The Netherlands 2010-2013. J Med Entomol 52, 185–198.

Ibañez-Justicia, A., Teekema, S., Den Hartog, W., Jacobs, F., Dik, M., Stroo, A., 2018. The Effectiveness of Asian Bush Mosquito (*Aedes japonicus japonicus*) Control Actions in Colonised Peri-urban Areas in the Netherlands. J Med Entomol 55, 673–680.

Ikegami, T., Makino, S., 2011. The Pathogenesis of Rift Valley Fever. Viruses 3, 493–519.

Ivanova, N. V., Dewaard, J.R., Hebert, P.D.N., 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. Mol Ecol Notes 6, 998–1002.

Iwamura, T., Guzman-Holst, A., Murray, K.A., 2020. Accelerating invasion potential of disease vector *Aedes aegypti* under climate change. Nat Commun 11, 2130.

Jansen, C., Ritchie, S., van den Hurk, A., 2013. The Role of Australian Mosquito Species in the Transmission of Endemic and Exotic West Nile Virus Strains. Int J Environ Res Public Health 10, 3735–3752.

Jansen, S., Lühken, R., Helms, M., Pluskota, B., Pfitzner, W.P., Oerther, S., Becker, N., Schmidt-Chanasit, J., Heitmann, A., 2022. Vector Competence of Mosquitoes from Germany for Sindbis Virus. Viruses 14, 2644.

Jenerette, G.D., Harlan, S.L., Stefanov, W.L., Martin, C.A., 2011. Ecosystem services and urban heat riskscape moderation: water, green spaces, and social inequality in Phoenix, USA. Ecol Appl 21, 2637–2651.

Jetten, T.H., Focks, D.A., 1997. Potential Changes in the Distribution of Dengue Transmission under Climate Warming. Am J Trop Med Hyg 57, 285–297.

Johnson, M.F., Gómez, A., Pinedo-Vasquez, M., 2008. Land use and mosquito diversity in the Peruvian Amazon. J Med Entomol 45, 1023–1030.

Johnson, M.T.J., Munshi-South, J., 2017. Evolution of life in urban environments. Science 358, eaam8327.

Johnson, P.H., Russell, R.C., 2019. Colonization of *Coquillettidia linealis* (Skuse) with reference to other *Coquillettidia* and *Mansonia* species. J Vector Ecol 44, 130–137.

Jourdain, E., Gauthier-Clerc, M., Bicout, D., Sabatier, P., 2007. Bird Migration Routes and Risk for Pathogen Dispersion into Western Mediterranean Wetlands. Emerg Infect Dis 13, 365–372.

Joyce, A.L., Melese, E., Ha, P.-T., Inman, A., 2018. Population genetic structure of the *Culex pipiens* (Diptera: Culicidae) complex, vectors of West Nile virus, in five habitats. Parasit Vectors 11, 10.

Jupp, P.G., 2001. The Ecology of West Nile Virus in South Africa and the Occurrence of Outbreaks in Humans. Ann N Y Acad Sci 951, 143–152.

Kache, P.A., Santos-Vega, M., Stewart-Ibarra, A.M., Cook, E.M., Seto, K.C., Diuk-Wasser, M.A., 2022. Bridging landscape ecology and urban science to respond to the rising threat of mosquito-borne diseases. Nat Ecol Evol 6, 1601–1616.

Kalan, K., Šušnjar, J., Ivović, V., Buzan, E., 2017. First record of *Aedes koreicus* (Diptera, Culicidae) in Slovenia. Parasitol Res 116, 2355–2358.

Kampen, H., Kronefeld, M., Zielke, D., Werner, D., 2013. Three rarely encountered and one new *Culiseta* species (Diptera: Culicidae) in Germany. J Eur Mosq Control Assoc 31, 36–39.

Kampen, H., Kuhlisch, C., Fröhlich, A., Scheuch, D.E., Walther, D., 2016a. Occurrence and Spread of the Invasive Asian Bush Mosquito *Aedes japonicus japonicus* (Diptera: Culicidae) in West and North Germany since Detection in 2012 and 2013, Respectively. PLoS One 11, e0167948.

Kampen, H., Medlock, J.M., Vaux, A.G.C., Koenraadt, C.J.M., Van Vliet, A.J.H., Bartumeus, F., Oltra, A., Sousa, C.A., Chouin, S., Werner, D., 2015. Approaches to passive mosquito surveillance in the EU. Parasit Vectors 8, 9.

Kampen, H., Schäfer, M., Zielke, D.E., Walther, D., 2016b. The *Anopheles maculipennis* complex (Diptera: Culicidae) in Germany: an update following recent monitoring activities. Parasitol Res 115, 3281–3294.

Kampen, H., Schuhbauer, A., Walther, D., 2017. Emerging mosquito species in Germany—a synopsis after 6 years of mosquito monitoring (2011–2016). Parasitol Res 116, 3253–3263.

Kampen, H., Walther, D., 2018. Vector Potential of Mosquito Species (Diptera: Culicidae) Occurring in Central Europe, in: Mosquito-Borne Diseases: Implications for Public Health. pp. 41–68.

Kampen, H., Werner, D., 2014. Out of the bush: the Asian bush mosquito *Aedes japonicus japonicus* (Theobald, 1901) (Diptera, Culicidae) becomes invasive. Parasit Vectors 7, 59.

Kantele, A., Jokiranta, T.S., 2011. Review of Cases with the Emerging Fifth Human Malaria Parasite, *Plasmodium knowlesi*. Clin Infect Dis 52, 1356–1362.

Kaplan, L., Kendell, D., Robertson, D., Livdahl, T., Khatchikian, C., 2010. *Aedes aegypti* and *Aedes albopictus* in Bermuda: extinction, invasion, invasion and extinction. Biol Invasions 12, 3277–3288.

Kaufman, M.G., Fonseca, D.M., 2014. Invasion Biology of *Aedes japonicus japonicus* (Diptera: Culicidae). Annu Rev Entomol 59, 31–49.

Kemenesi, G., Dallos, B., Oldal, M., Kutas, A., Földes, F., Németh, V., Reiter, P., Bakonyi, T., Bányai, K., Jakab, F., 2014. Putative novel lineage of West Nile virus in *Uranotaenia unguiculata* mosquito, Hungary. Virusdisease 25, 500–503.

Kenea, O., Balkew, M., Tekie, H., Gebre-Michael, T., Deressa, W., Loha, E., Lindtjørn, B., Overgaard, H.J., 2017. Comparison of two adult mosquito sampling methods with human landing catches in south-central Ethiopia. Malar J 16, 30.

Kerkow, A., Wieland, R., Früh, L., Hölker, F., Jeschke, J.M., Werner, D., Kampen, H., 2020. Can data from native mosquitoes support determining invasive species habitats? Modelling the climatic niche of *Aedes japonicus* japonicus (Diptera, Culicidae) in Germany. Parasitol Res 119, 31–42.

Kilpatrick, A.M., Daszak, P., Goodman, S.J., Rogg, H., Kramer, L.D., Cedeño, V., Cunningham, A.A., 2006. Predicting pathogen introduction: West nile virus spread to Galápagos. Conserv Biol 20, 1224–1231.

Kilpatrick, A.M., Kramer, L.D., Jones, M.J., Marra, P.P., Daszak, P., Fonseca, D.M., 2007. Genetic Influences on Mosquito Feeding Behavior and the Emergence of Zoonotic Pathogens. Am J Trop Med Hyg 77, 667–671.

Kilpatrick, A.M., Randolph, S.E., 2012. Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. Lancet 380, 1946–1955.

Kim, S., Trocke, S., Sim, C., 2018. Comparative studies of stenogamous behaviour in the mosquito *Culex pipiens* complex. Med Vet Entomol 32, 427–435.

Kline, D.L., Patnaude, M., Barnard, D.R., 2006. Efficacy of four trap types for detecting and monitoring *Culex* spp. in north central Florida. J Med Entomol 43, 1121–1128.

Knapp, A.K., Hoover, D.L., Wilcox, K.R., Avolio, M.L., Koerner, S.E., La Pierre, K.J., Loik, M.E., Luo, Y., Sala, O.E., Smith, M.D., 2015. Characterizing differences in precipitation regimes of extreme wet and dry years: implications for climate change experiments. Glob Chang Biol 21, 2624–2633.

Knight, K.L., Stone, A., 1959. A Catalog of the Mosquitoes of the World (Diptera: Culicidae), A Catalog of the Mosquitoes of the World (Diptera: Culicidae). SPIE.

Koban, M.B., Kampen, H., Scheuch, D.E., Frueh, L., Kuhlisch, C., Janssen, N., Steidle, J.L.M., Schaub, G.A., Werner, D., 2019. The Asian bush mosquito *Aedes japonicus japonicus* (Diptera: Culicidae) in Europe, 17 years after its first detection, with a focus on monitoring methods. Parasit Vectors 12, 109.

Koenraadt, C.J.M., Harrington, L.C., 2008. Flushing effect of rain on container-inhabiting mosquitoes *Aedes aegypti* and *Culex pipiens* (Diptera: Culicidae). J Med Entomol 45, 28–35.

Krol, L., Langezaal, M., Budidarma, L., Wassenaar, D., Didaskalou, E.A., Trimbos, K., Dellar, M., van Bodegom, P.M., Geerling, G.W., Schrama, M., 2024. Distribution of *Culex pipiens* life stages across urban green and grey spaces in Leiden, The Netherlands. Parasit Vectors 17, 37.

Krüger, A., Börstler, J., Badusche, M., Lühken, R., Garms, R., Tannich, E., 2014. Mosquitoes (Diptera: Culicidae) of metropolitan Hamburg, Germany. Parasitol Res 113, 2907–2914.

Krüger, A., Rech, A., Su, X., Tannich, E., 2001. Two cases of autochthonous *Plasmodium falciparum* malaria in Germany with evidence for local transmission by indigenous *Anopheles plumbeus*. Trop Med Int Heal 6, 983–985.

Kubica-Biernat, B., 1999. Distribution of mosquitoes (Diptera: Culicidae) in Poland. Eur Mosq Bull 5, 1–17.

Kurucz, K., Kemenesi, G., Zana, B., Zeghbib, S., Oldal, M., Jakab, F., 2017. Ecological preferences of the putative West Nile virus vector *Uranotaenia unguiculata* mosquito with description of an original larval habitat. North West J Zool 13, 193–199.

Kurucz, K., Kiss, V., Zana, B., Schmieder, V., Kepner, A., Jakab, F., Kemenesi, G., 2016. Emergence of *Aedes koreicus* (Diptera: Culicidae) in an urban area, Hungary, 2016. Parasitol Res 115, 4687–4689.

La Ruche, G., Souarès, Y., Armengaud, A., Peloux-Petiot, F., Delaunay, P., Desprès, P., Lenglet, A., Jourdain, F., Leparc-Goffart, I., Charlet, F., Ollier, L., Mantey, K., Mollet, T., Fournier, J.P., Torrents, R., Leitmeyer, K., Hilairet, P., Zeller, H., Van Bortel, W., Dejour-Salamanca, D., Grandadam, M., Gastellu-Etchegorry, M., 2010. First two autochthonous dengue virus infections in metropolitan France, September 2010. Euro Surveill 15, 19676.

LaDeau, S.L., Allan, B.F., Leisnham, P.T., Levy, M.Z., 2015. The ecological foundations of transmission potential and vector-borne disease in urban landscapes. Funct Ecol 29, 889–901.

LaDeau, S.L., Calder, C.A., Doran, P.J., Marra, P.P., 2011. West Nile virus impacts in American crow populations are associated with human land use and climate. Ecol Res 26, 909–916.

Laird, M., Calder, L., Thornton, R.C., Syme, R., Holder, P.W., Mogi, M., 1994. Japanese *Aedes albopictus* among four mosquito species reaching New Zealand in used tires. J Am Mosq Control Assoc 10, 14–23.

Lapointe, D.A., 2008. Dispersal of *Culex quinquefasciatus* (Diptera: Culicidae) in a Hawaiian rain forest. J Med Entomol 45, 600–609.

Lazzarini, L., Barzon, L., Foglia, F., Manfrin, V., Pacenti, M., Pavan, G., Rassu, M., Capelli, G., Montarsi, F., Martini, S., Zanella, F., Padovan, M.T., Russo, F., Gobbi, F., 2020. First autochthonous dengue outbreak in Italy, August 2020. Euro Surveill 25, 2001606.

Lebl, K., Zittra, C., Silbermayr, K., Obwaller, A., Berer, D., Brugger, K., Walter, M., Pinior, B., Fuehrer, H.-P., Rubel, F., 2015. Mosquitoes (Diptera: Culicidae) and their relevance as disease vectors in the city of Vienna, Austria. Parasitol Res 114, 707–713.

Lehane, M.J., 2005. The Biology of Blood-Sucking in Insects, The Biology of Blood-Sucking in Insects, Second Edition. Cambridge University Press, Cambridge.

Lewis, S.L., Maslin, M.A., 2015. Defining the Anthropocene. Nature 519, 171–180.

Li, Y., Kamara, F., Zhou, G., Puthiyakunnon, S., Li, C., Liu, Y., Zhou, Y., Yao, L., Yan, G., Chen, X.-G., 2014. Urbanization Increases *Aedes albopictus* Larval Habitats and Accelerates Mosquito Development and Survivorship. PLoS Negl Trop Dis 8, e3301.

Lindström, A., Lilja, T., 2018. First finding of the West Nile virus vector *Culex modestus* Ficalbi 1889 (Diptera; Culicidae) in Sweden. J Eur Mosq Control Assoc 36, 1–2.

Loibl, W., Ravetz, J., Tosics, I., 2011. Concepts and methods, in: Piorr, A., Ravetz, J., Tosics, I. (Eds.), Peri-Urbanisation in Europe. University of Copenhagen/Academic Books Life Sciences, Copenhagen, pp. 24–29.

López-Ruiz, N., Montaño-Remacha, M. del C., Durán-Pla, E., Pérez-Ruiz, M., Navarro-Marí, J.M., Salamanca-Rivera, C., Miranda, B., Oyonarte-Gómez, S., Ruiz-Fernández, J., 2018. West Nile virus outbreak in humans and epidemiological surveillance, west Andalusia, Spain, 2016. Euro Surveill 23, 17–00261.

Lorenz, A.R., Walker, E.D., Kaufman, M.G., 2013. Does Autocthonous Primary Production Influence Oviposition by *Aedes japonicus japonicus* (Diptera: Culicidae) in Container Habitats? J Med Entomol 50, 69–78.

Lounibos, L.P., Bargielowski, I., Carrasquilla, M.C., Nishimura, N., 2016. Coexistence of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in Peninsular Florida Two Decades After Competitive Displacements. J Med Entomol 53, 1385–1390.

Lounibos, L.P., Escher, R.L., Lourenço-De-Oliveira, R., 2003. Asymmetric evolution of photoperiodic diapause in temperate and tropical invasive populations of *Aedes albopictus* (Diptera: Culicidae). Ann Entomol Soc Am 96, 512–518.

Lounibos, L.P., Juliano, S.A., 2018. Where vectors collide: the importance of mechanisms shaping the realized niche for modeling ranges of invasive *Aedes* mosquitoes. Biol Invasions 20, 1913–1929.

Lucientes-Curdi, J., Molina-Moreno, R., Amela-Heras, C., Simon-Soria, F., Santos-Sanz, S., Sánchez-Gómez, A., Suárez-Rodriguez, B., Sierra-Moros, M.J., 2014. Dispersion of *Aedes albopictus* in the Spanish Mediterranean Area. Eur J Public Health 24, 637–40.

Lühken, R., Brattig, N., Becker, N., 2023. Introduction of invasive mosquito species into Europe and prospects for arbovirus transmission and vector control in an era of globalization. Infect Dis Poverty 12, 109.

Lühken, R., Czajka, C., Steinke, S., Jöst, H., Schmidt-Chanasit, J., Pfitzner, W., Becker, N., Kiel, E., Krüger, A., Tannich, E., 2016. Distribution of individual members of the mosquito *Anopheles maculipennis* complex in Germany identified by newly developed real-time PCR assays. Med Vet Entomol 30, 144–154.

Lühken, R., Steinke, S., Leggewie, M., Tannich, E., Krüger, A., Becker, S., Kiel, E., 2015. Physico-Chemical Characteristics of *Culex pipiens* sensu lato and *Culex torrentium* (Diptera: Culicidae) Breeding Sites in Germany: Table 1. J Med Entomol 52, 932–936.

Lundström, J., 1994. Vector competence of Western European mosquitoes for arboviruses: A review of field and experimental studies. Bull Soc Vector Ecol 19, 23–36.

Lundström, J., Schäfer, M., Hesson, J.C., Blomgren, E., Lindstrom, A., Wahlqvist, P., Halling, A., Hagelin, A., Ahlm, C., Evander, M., Broman, T., Forsman, M., Persson Vinnersten, T.Z., 2013. The geographic distribution of mosquito species in Sweden. J Eur Mosq Control Assoc 31, 21–35.

Maliti, D. V., Govella, N.J., Killeen, G.F., Mirzai, N., Johnson, P.C.D., Kreppel, K., Ferguson, H.M., 2015. Development and evaluation of mosquito-electrocuting traps as alternatives to the human landing catch technique for sampling host-seeking malaria vectors. Malar J 14, 502.

Malkinson, M., Banet, C., 2002. The role of birds in the ecology of West Nile virus in Europe and Africa. Curr Top Microbiol Immunol 267, 309–22.

Mancini, G., Montarsi, F., Calzolari, M., Capelli, G., Dottori, M., Ravagnan, S., Lelli, D., Chiari, M., Santilli, A., Quaglia, M., Federici, V., Monaco, F., Goffredo, M., Savini, G., 2017. Mosquito species involved in the circulation of West Nile and Usutu viruses in Italy. Vet Ital 53, 97–110.

Manguin, S., Boete, C., 2011. Global Impact of Mosquito Biodiversity, Human Vector-Borne Diseases and Environmental Change, in: The Importance of Biological Interactions in the Study of Biodiversity. InTech, pp. 27–50.

Marini, G., Arnoldi, D., Baldacchino, F., Capelli, G., Guzzetta, G., Merler, S., Montarsi, F., Rizzoli, A., Rosà, R., 2019. First report of the influence of temperature on the bionomics and population dynamics of *Aedes koreicus*, a new invasive alien species in Europe. Parasit Vectors 12, 524.

Marini, G., Guzzetta, G., Baldacchino, F., Arnoldi, D., Montarsi, F., Capelli, G., Rizzoli, A., Merler, S., Rosà, R., 2017. The effect of interspecific competition on the temporal dynamics of *Aedes albopictus* and *Culex pipiens*. Parasit Vectors 10, 102.

Marquetti, M.C., Valdés, V., Aguilera, L., Navarro, A., 2000. Entomological surveillance over *Aedes* (S) *aegypti* and other culicids in Ciudad de La Habana, Cuba 1991-1996. Rev Cubana Med Trop 52, 133–137.

Martens, P., Hall, L., 2000. Malaria on the move: human population movement and malaria transmission. Emerg Infect Dis 6, 103.

Martinet, J.-P., Ferté, H., Failloux, A.-B., Schaffner, F., Depaquit, J., 2019. Mosquitoes of North-Western Europe as Potential Vectors of Arboviruses: A Review. Viruses 11, 1059.

Martinet, J.-P., Ferté, H., Sientzoff, P., Krupa, E., Mathieu, B., Depaquit, J., 2021. Wing Morphometrics of *Aedes* Mosquitoes from North-Eastern France. Insects 12, 341.

Martínez de la Puente, J., Dunn, J.C., Gangoso, L., 2021. Editorial: Factors Affecting Host Selection by Mosquitoes: Implications for the Transmission of Vector-Borne Pathogens. Front Ecol Evol 9, 739258.

Martínez de la Puente, J., Ferraguti, M., Ruiz, S., Roiz, D., Soriguer, R.C., Figuerola, J., 2016. *Culex pipiens* forms and urbanization: effects on blood feeding sources and transmission of avian *Plasmodium*. Malar J 15, 589.

Martínez de la Puente, J., Moreno-Indias, I., Hernández-Castellano, L.E., Argüello, A., Ruiz, S., Soriguer, R., Figuerola, J., 2012. Host-Feeding Pattern of *Culex theileri* (Diptera: Culicidae), Potential Vector of *Dirofilaria immitis* in the Canary Islands, Spain. J Med Entomol 49, 1419–1423.

Martínez de la Puente, J., Ruiz, S., Soriguer, R., Figuerola, J., 2013. Effect of blood meal digestion and DNA extraction protocol on the success of blood meal source determination in the malaria vector *Anopheles atroparvus*. Malar J 12, 109.

Martínez de la Puente, J., Soriguer, R., Senar, J.C., Figuerola, J., Bueno-Mari, R., Montalvo, T., 2020. Mosquitoes in an Urban Zoo: Identification of Blood Meals, Flight Distances of Engorged Females, and Avian Malaria Infections. Front Vet Sci 7, 460.

Martínez-Barciela, Y., González, A.P., Rial, D.G., González, J.G., 2021. First records of five species of mosquitoes (Diptera: Culicidae) in Galicia, including the first evidence of the genus *Coquillettidia* in northwestern Spain. J Vector Ecol 46, 96–102.

Martínez-Barciela, Y., Martínez, J.M.P., Torres, M.I.S., Ortega, Á.P., González, J.C.O., González, J.G., 2020. First records of *Anopheles (Anopheles) plumbeus* Stephens, 1828 and *Culex (Culex) torrentium* Martini, 1925 (Diptera: Culicidae) in Galicia (NW Spain). J Vector Ecol 45, 306–311.

Martínez-Barciela, Y., Polina, A., Garrido, J., 2024. Ecology and diversity of mosquito larvae in ponds and lagoons of Northwestern Spain. Med Vet Entomol 38, 38–47.

McKinney, M., 2002. Urbanization, Biodiversity, and Conservation The impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population. Bioscience 52, 883–890.

McKinney, M.L., 2008. Effects of urbanization on species richness: A review of plants and animals. Urban Ecosyst 11, 161–176.

McKinney, M.L., 2006. Urbanization as a major cause of biotic homogenization. Biol Conserv 127, 247–260.

McNelly, J.R., 1989. The CDC trap as a special monitoring tool. Proceedings Annual Meeting, New Jersey Mosquito Control Association, Inc 76, 26–33.

Medeiros-Sousa, A.R., Ceretti-Júnior, W., de Carvalho, G.C., Nardi, M.S., Araujo, A.B., Vendrami, D.P., Marrelli, M.T., 2015. Diversity and abundance of mosquitoes (Diptera:Culicidae) in an urban park: Larval habitats and temporal variation. Acta Trop 150, 200–209.

Medeiros-Sousa, A.R., Fernandes, A., Ceretti-Junior, W., Wilke, A.B.B., Marrelli, M.T., 2017. Mosquitoes in urban green spaces: using an island biogeographic approach to identify drivers of species richness and composition. Sci Rep 7, 17826.

Medlock, J.M., Hansford, K.M., Anderson, M., Mayho, R., Snow, K.R., 2012. Mosquito nuisance and control in the UK - a questionnaire-based survey of local authorities. Eur Mosq Bull 15–29.

Medlock, J.M., Hansford, K.M., Schaffner, F., Versteirt, V., Hendrickx, G., Zeller, H., Bortel, W. Van, 2012. A review of the invasive mosquitoes in Europe: Ecology, public health risks, and control options. Vector-Borne Zoonotic Dis 12, 435–447.

Medlock, J.M., Hansford, K.M., Versteirt, V., Cull, B., Kampen, H., Fontenille, D., Hendrickx, G., Zeller, H., Van Bortel, W., Schaffner, F., 2015. An entomological review of invasive mosquitoes in Europe. Bull Entomol Res 105, 637–663.

Medlock, Jolyon M, Vaux, A., 2015. Impacts of the creation, expansion and management of English wetlands on mosquito presence and abundance – developing strategies for future disease mitigation. Parasit Vectors 8, 142.

Medlock, Jolyon M., Vaux, A.G.C., 2015. Seasonal dynamics and habitat specificity of mosquitoes in an English wetland: implications for UK wetland management and restoration. J Vector Ecol 40, 90–106.

Medlock, J.M., Vaux, A.G.C., 2013. Colonization of UK coastal realignment sites by mosquitoes: implications for design, management, and public health. J Vector Ecol 38, 53–62.

Mehlhorn, H., 2016. Mosquitoes, in: Encyclopedia of Parasitology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 1699–1706.

Mereta, S.T., Yewhalaw, D., Boets, P., Ahmed, A., Duchateau, L., Speybroeck, N., Vanwambeke, S.O., Legesse, W., De Meester, L., Goethals, P.L., 2013. Physico-chemical and biological characterization of anopheline mosquito larval habitats (Diptera: Culicidae): implications for malaria control. Parasit Vectors 6, 320.

Merritt, R.W., Dadd, R.H., Walker, E.D., 1992. Feeding Behavior, Natural Food, and Nutritional Relationships of Larval Mosquitoes. Annu Rev Entomol 37, 349–374.

Meyerson, L.A., Mooney, H.A., 2007. Invasive alien species in an era of globalization. Front Ecol Environ 5, 199–208.

Milankov, V., Petrić, D., Vujić, A., Vapa, L., 2009. Taxonomy, Biology, Genetic Variability and Medical Importance of *Ochlerotatus caspius* (Pallas, 1771) and *O. dorsalis* (Meigen, 1830) (Diptera: Culicidae). Acta Entomol Serbica 14, 195–207.

Minakawa, N., Githure, J.I., Beier, J.C., Yan, G., 2001. Anopheline Mosquito Survival Strategies During the Dry Period in Western Kenya. J Med Entomol 38, 388–392.

Ministerio de Sanidad, 2023. Plan Nacional de Prevención, Vigilancia y Control de las enfermedades transmitidas por vectores. Parte I. Enfermedades transmitidas por *Aedes*. Parte II: Enfermedades transmitidas por *Culex*. Abril 2023 [WWW Document]. URL https://www.sanidad.gob.es/profesionales/saludPublica/ccayes/activPreparacionRespuesta/doc/PLAN_DE_VECTORES.pdf (accessed 5.1.24).

Miranda, M.Á., Barceló, C., Arnoldi, D., Augsten, X., Bakran-Lebl, K., Balatsos, G., Bengoa, M., Bindler, P., Boršová, K., Bourquia, M., Bravo-Barriga, D., Čabanová, V., Caputo, B., Christou, M., Delacour, S., Eritja, R., Fassi-Fihri, O., Ferraguti, M., Flacio, E., Frontera, E., Fuehrer, H.-P., García-Pérez, A.L., Georgiades, P., Gewehr, S., Goiri, F., González, M.A., Gschwind, M., Gutiérrez-López, R., Horváth, C., Ibáñez-Justicia, A., Jani, V., Kadriaj, P., Kalan, K., Kavran, M., Klobucar, A., Kurucz, K., Lucientes, J., Lühken, R., Magallanes, S., Marini, G., Martinou, A.F., Michelutti, A., Mihalca, A.D., Montalvo, T., Montarsi, F., Mourelatos, S., Muja-Bajraktari, N., Müller, P., Notarides, G., Osório, H.C., Oteo, J.A., Oter, K., Pajović, I., Palmer, J.R.B., Petrinic, S., Răileanu, C., Ries, C., Rogozi, E., Ruiz-Arrondo, I., Sanpera-Calbet, I., Sekulić, N., Sevim, K., Sherifi, K., Silaghi, C., Silva, M., Sokolovska, N., Soltész, Z., Sulesco, T., Šušnjar, J., Teekema, S., Valsecchi, A., Vasquez, M.I., Velo, E., Michaelakis, A., Wint, W., Petrić, D., Schaffner, F., della Torre, A., 2022. AIMSurv: First pan-European harmonized surveillance of *Aedes* invasive mosquito species of relevance for human vector-borne diseases. GigaByte 2022.

Miró, G., Montoya, A., Roura, X., Gálvez, R., Sainz, A., 2013. Seropositivity rates for agents of canine vector-borne diseases in Spain: a multicentre study. Parasit Vectors 6, 117.

Mixão, V., Bravo Barriga, D., Parreira, R., Novo, M.T., Sousa, C.A., Frontera, E., Venter, M., Braack, L., Almeida, A.P.G., 2016. Comparative morphological and molecular analysis confirms the presence of the West Nile virus mosquito vector, *Culex univittatus*, in the Iberian Peninsula. Parasit Vectors 9, 601.

Mogi, M., Armbruster, P.A., Tuno, N., Wilkerson, R., 2020. Differences in Responses to Urbanization between Invasive Mosquitoes, *Aedes japonicus japonicus* (Diptera: Culicidae) and *Aedes albopictus*, in Their Native Range, Japan. J Med Entomol 57, 104–112.

Möhlmann, T.W.R., Wennergren, U., Tälle, M., Favia, G., Damiani, C., Bracchetti, L., Koenraadt, C.J.M., 2017. Community analysis of the abundance and diversity of mosquito species (Diptera: Culicidae) in three European countries at different latitudes. Parasit Vectors 10, 510.

Monge, S., García-Ortúzar, V., López Hernández, B., Lopaz Pérez, M.Á., Delacour-Estrella, S., Sánchez-Seco, M.P., Fernández Martinez, B., García San Miguel, L., García-Fulgueiras, A., Sierra Moros, M.J., 2020. Characterization of the first autochthonous dengue outbreak in Spain (August–September 2018). Acta Trop 205, 105402.

Montarsi, F., Drago, A., Russo, F., 2014. Current Knowledge on the Distribution and Biology of the Recently Introduced Invasive Mosquito *Aedes koreicus* (Diptera: Culicidae). Atti Accad Naz Ital Entomol 62, 169–174.

Montarsi, F., Martini, S., Michelutti, A., Da Rold, G., Mazzucato, M., Qualizza, D., Di Gennaro, D., Di Fant, M., Dal Pont, M., Palei, M., Capelli, G., 2019. The invasive mosquito *Aedes japonicus japonicus* is spreading in northeastern Italy. Parasit Vectors 12, 120.

Montgomery, M.J., Thiemann, T., Macedo, P., Brown, D.A., Scott, T.W., 2011. Blood-Feeding Patterns of the *Culex pipiens* Complex in Sacramento and Yolo Counties, California. J Med Entomol 48, 398–404.

Moore, C., 1997. *Aedes albopictus* in the United States: Ten-Year Presence and Public Health Implications. Emerg Infect Dis 3, 329–334.

Moore, S.J., Zunwei, D., Hongning, Z., Xuezhong, W., Hongbing, L., Yujiang, X., Hill, N., 2001. The efficacy of different mosquito trapping methods in a forest-fringe village, Yunnan Province, southern China. Southeast Asian J Trop Med Pub Hlth 32, 282–289.

Mora, C., McKenzie, T., Gaw, I.M., Dean, J.M., von Hammerstein, H., Knudson, T.A., Setter, R.O., Smith, C.Z., Webster, K.M., Patz, J.A., Franklin, E.C., 2022. Over half of known human pathogenic diseases can be aggravated by climate change. Nat Clim Change 12, 869–875.

Mora-Rubio, C., Ferraguti, M., Magallanes, S., Bravo-Barriga, D., Hernandez-Caballero, I., Marzal, A., de Lope, F., 2023. Unravelling the mosquito-haemosporidian parasite-bird host network in the southwestern Iberian Peninsula: insights into malaria infections, mosquito community and feeding preferences. Parasit Vectors 16, 395.

Morchón, R., Bargues, M.D., Latorre, J.M., Melero-Alcíbar, R., Pou-Barreto, C., Mas-Coma, S., Simón, F., 2007. Haplotype H1 of *Culex pipiens* Implicated as Natural Vector of *Dirofilaria immitis* in an Endemic Area of Western Spain. Vector-Borne Zoonotic Dis 7, 653–658.

Morchón R., Bargues, M.D., Latorre-Estivalis, J.M., Pou-Barreto, C., Melero-Alcibar, R., Moreno, M., Valladares, B., Molina, R., Montoya Alonso, J.A., Mas-Coma, S., Simón, F., 2013. Molecular Characterization of *Culex theileri* from Canary Islands, Spain, a Potential Vector of *Dirofilaria immitis*. J Clin Exp Pathol S3:001.

Mori, A., Oda, T., Wada, Y., 1981. Studies on the egg diapause and overwintering of *Aedes albopictus* in Nagasaki. Trop Med 23, 79–90.

Mosquitoweb Difenditi Dalle Zanzare, 2022. *Ochlerotatus rusticus* [WWW Document]. URL https://www.mosquitoweb.it/blog/post/ochlerotatus-rusticus.html (accessed 5.1.24).

Müller, G.C., Kravchenko, V.D., Junnila, A., Schlein, Y., 2012. Tree-hole breeding mosquitoes in Israel. J Vector Ecol 37, 102–109.

Müller, P., Engeler, L., Vavassori, L., Suter, T., Guidi, V., Gschwind, M., Tonolla, M., Flacio, E., 2020. Surveillance of invasive *Aedes* mosquitoes along Swiss traffic axes reveals different dispersal modes for *Aedes albopictus* and *Ae. japonicus*. PLoS Negl Trop Dis 14, e0008705.

Muñoz, J., Eritja, R., Alcaide, M., Montalvo, T., Soriguer, R.C., Figuerola, J., 2011. Host-Feeding Patterns of Native *Culex pipiens* and Invasive *Aedes albopictus* Mosquitoes (Diptera: Culicidae) in Urban Zones from Barcelona, Spain. J Med Entomol 48, 956–960.

Muñoz, J., Ruiz, S., Soriguer, R., Alcaide, M., Viana, D.S., Roiz, D., Vázquez, A., Figuerola, J., 2012. Feeding Patterns of Potential West Nile Virus Vectors in South-West Spain. PLoS One 7, e39549.

Murgue, B., Murri, S., Zientara, S., Durand, B., Durand, J.P., Zeller, H., 2001. West Nile outbreak in horses in southern France, 2000: the return after 35 years. Emerg Infect Dis 7, 692.

Muturi, E.J., Mwangangi, J., Shililu, J., Jacob, B.G., Mbogo, C., Githure, J., Novak, R.J., 2008. Environmental factors associated with the distribution of *Anopheles arabiensis* and *Culex quinquefasciatus* in a rice agro-ecosystem in Mwea, Kenya. J Vector Ecol 33, 56–63.

Nascimento, K.L.C., Silva, J.F.M. da, Zequi, J.A.C., Lopes, J., 2020. Comparison Between Larval Survey Index and Positive Ovitrap Index in the Evaluation of Populations of *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762) North of Paraná, Brazil. Environ Health Insights 14.

Nava, A., Shimabukuro, J.S., Chmura, A.A., Luz, S.L.B., 2017. The Impact of Global Environmental Changes on Infectious Disease Emergence with a Focus on Risks for Brazil. ILAR J 58, 393

Nawrocki, S.J., Craig, G.B., 1989. Further extension of the range of the rock pool mosquito, *Aedes atropalpus*, via tire breeding. J Am Mosq Control Assoc 5, 110–114.

Nayar, J.K., Sauerman, D.M., 1977. The effects of nutrition on survival and fecundity in Florida mosquitoes. Part 2. Utilization of a blood meal for survival. J Med Entomol 12, 99–103.

Novikov, Y.M., Vaulin, O. V, 2014. Expansion of *Anopheles maculipennis* s.s. (Diptera: Culicidae) to northeastern Europe and northwestern Asia: Causes and Consequences. Parasit Vectors 7, 389.

O'Hara, R.B., Kotze, D.J., 2010. Do not log-transform count data. Methods Ecol Evol 1, 118–122.

Oke, T.R., 1973. City size and the urban heat island. Atmospheric Environment (1967) 7, 769–779.

Oksanen, J., Simpson Gavin, Blanchet F, Kindt Roeland, Legendre Pierre, Minchin Peter, O'Hara R.B., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour Matt, Bedward Michael, Bolker Ben, Borcard Daniel, Carvalho Gustavo, Chirico Michael, De Caceres Miquel, Durand Sebastien, Evangelista Heloisa, FitzJohn Rich, Friendly Michael, Furneaux Brendan, Hannigan Geoffrey, Hill Mark O, Lahti Leo, McGlinn Dan, Ouellette Marie-Helene, Ribeiro Cunha Eduardo, Smith Tyler, Stier Adrian, Ter Braak Cajo, Weedon James, 2022. vegan: Community Ecology Package. R package version 2.6-4. Community ecology package 2.6-4.

Olaso, A., Ramos, J.M., López-Ballero, M.F., Olaso, I., 2017. Malaria in Europe: Follow-up of autochthonous malaria in Greece and new risks. Enferm Infecc Microbiol Clin 35, 543–544.

Osório, H.C., Zé-Zé, L., Alves, M.J., 2012. Host-Feeding Patterns of *Culex pipiens* and Other Potential Mosquito Vectors (Diptera: Culicidae) of West Nile Virus (*Flaviviridae*) Collected in Portugal. J Med Entomol 49, 717–721.

Osório, H.C., Zé-Zé, L., Amaro, F., Nunes, A., Alves, M.J., 2014. Sympatric occurrence of *Culex pipiens* (Diptera, Culicidae) biotypes pipiens, molestus and their hybrids in Portugal, Western Europe: feeding patterns and habitat determinants. Med Vet Entomol 28, 103–109.

Otranto, D., Dantas-Torres, F., 2010. Canine and feline vector-borne diseases in Italy: current situation and perspectives. Parasit Vectors 3.

Paaijmans, K.P., Imbahale, S.S., Thomas, M.B., Takken, W., 2010. Relevant microclimate for determining the development rate of malaria mosquitoes and possible implications of climate change. Malar J 9, 196.

Pachler, K., Lebl, K., Berer, D., Rudolf, I., Hubalek, Z., Nowotny, N., 2014. Putative New West Nile Virus Lineage in *Uranotaenia unguiculata* Mosquitoes, Austria, 2013. Emerg Infect Dis 20, 2119–2122.

Patz, J.A., Daszak, P., Tabor, G.M., Aguirre, A.A., Pearl, M., Epstein, Jon, Wolfe, N.D., Kilpatrick, A.M., Foufopoulos, J., Molyneux, David, Bradley, David J., Amerasinghe, F.P., Ashford, R.W., Barthelemy, D., Bos, R., Bradley, D. J., Buck, A., Butler, C., Chivian, E.S., Chua, K.B., Clark, G., Colwell, R., Confalonieri, U.E., Corvalan, C., Cunningham, A.A., Dein, J., Dobson, A.P., Else, J.G., Epstein, J., Field, H., Furu, P., Gascon, C., Graham, D., Haines, A., Hyatt, A.D., Jamaluddin, A., Kleinau, E.F., Koontz, F., Koren, H.S., LeBlancq, S., Lele, S., Lindsay, S., Maynard, N., McLean, R.G., McMichael, T., Molyneux, D., Morse, S.S., Norris, D.E., Ostfeld, R.S., Pearl, M.C., Pimentel, D., Rakototiana, L., Randriamanajara, O., Riach, J., Rosenthal, J.P., Salazar-Sanchez, E., Silbergeld, E., Thomson, M., Vittor, A.Y., Yameogo, L., Zakarov, V., 2004. Unhealthy landscapes: Policy recommendations on land use change and infectious disease emergence. Environ Health Perspect 112, 1092–1098.

Paull, S.H., Horton, D.E., Ashfaq, M., Rastogi, D., Kramer, L.D., Diffenbaugh, N.S., Kilpatrick, A.M., 2017. Drought and immunity determine the intensity of West Nile virus epidemics and climate change impacts. Proc R Soc B 284, 20162078.

Paupy, C., Delatte, H., Bagny, L., Corbel, V., Fontenille, D., 2009. *Aedes albopictus*, an arbovirus vector: From the darkness to the light. Microbes Infect 11, 1177–1185.

Paz, S., 2015. Climate change impacts on West Nile virus transmission in a global context. Philos Trans R Soc B Biol Sci 370, 20130561.

Perrin, A., Glaizot, O., Christe, P., 2022. Worldwide impacts of landscape anthropization on mosquito abundance and diversity: A meta-analysis. Glob Chang Biol 28, 6857–6871.

Peterson, A.T., 2008. Biogeography of diseases: a framework for analysis. Naturwissenschaften 95, 483–491.

Petrić, D., Petrović, T., Hrnjaković Cvjetković, I., Zgomba, M., Milošević, V., Lazić, G., Ignjatović Ćupina, A., Lupulović, D., Lazić, S., Dondur, D., Vaselek, S., Živulj, A., Kisin, B., Molnar, T., Janku, D., Pudar, D., Radovanov, J., Kavran, M., Kovačević, G., Plavšić, B., Jovanović Galović, A., Vidić, M., Ilić, S., Petrić, M., 2017. West Nile virus ‘circulation’ in Vojvodina, Serbia: Mosquito, bird, horse and human surveillance. Mol Cell Probes 31, 28–36.

Pimenta, P.F., Touray, M., Miller, L., 1994. The Journey of Malaria Sporozoites in the Mosquito Salivary Gland. J Euk Microbiol 41, 608–624.

Piperaki, E.T., Daikos, G.L., 2016. Malaria in Europe: emerging threat or minor nuisance? Clin Microbiol Infect 22, 487–493.

Platt, K.B., Tucker, B.J., Halbur, P.G., Tiawsirisup, S., Blitvich, B.J., Fabiosa, F.G., Bartholomay, L.C., Rowley, W.A., 2007. West Nile Virus Viremia in Eastern Chipmunks (*Tamias striatus*) Sufficient for Infecting Different Mosquitoes. Emerg Infect Dis 13, 831–837.

Poh, K.C., Chaves, L.F., Reyna-Nava, M., Roberts, C.M., Fredregill, C., Bueno, R., Debboun, M., Hamer, G.L., 2019. The influence of weather and weather variability on mosquito abundance and infection with West Nile virus in Harris County, Texas, USA. Sci Total Environ 675, 260–272.

Ponçon, N., Balenghien, T., Toty, C., Ferré, J.B., Thomas, C., Dervieux, A., L'Ambert, G., Schaffner, F., Bardin, O., Fontenille, D., 2007. Effects of Local Anthropogenic Changes on Potential Malaria Vector *Anopheles hyrcanus* and West Nile Virus Vector *Culex modestus,* Camargue, France. Emerg Infect Dis 13, 1810–1815.

Powell, J.R., Tabachnick, W.J., 2013. History of domestication and spread of *Aedes aegypti* - A Review. Mem Inst Oswaldo Cruz 108, 11–17.

Proft, J., Schaffner, F., Maier, W.A., Seitz, H.M., Kampem, H., Stermberg, A., Bastiam, S., 2003. Polymerase chain reaction-based differentiation of the mosquito sibling species *Anopheles claviger* ss and *Anopheles petragnani* (Diptera: Culicidae). Am J Trop Med Hyg 69, 195–199.

Prudhomme, J., Fontaine, A., Lacour, G., Gantier, J.-C., Diancourt, L., Velo, E., Bino, S., Reiter, P., Mercier, A., 2019. The native European *Aedes geniculatus* mosquito species can transmit chikungunya virus. Emerg Microbes Infect 8, 962–972.

Pyšek, P., Hulme, P.E., Simberloff, D., Bacher, S., Blackburn, T.M., Carlton, J.T., Dawson, W., Essl, F., Foxcroft, L.C., Genovesi, P., Jeschke, J.M., Kühn, I., Liebhold, A.M., Mandrak, N.E., Meyerson, L.A., Pauchard, A., Pergl, J., Roy, H.E., Seebens, H., van Kleunen, M., Vilà, M., Wingfield, M.J., Richardson, D.M., 2020. Scientists' warning on invasive alien species. Biol Rev 95, 1511–1534.

R Core Team, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Radrova, J., Seblova, V., Votypka, J., 2013. Feeding Behavior and Spatial Distribution of *Culex* Mosquitoes (Diptera: Culicidae) in Wetland Areas of the Czech Republic. J Med Entomol 50, 1097–1104.

Randolph, S.E., Rogers, D.J., 2010. The arrival, establishment and spread of exotic diseases: patterns and predictions. Nat Rev Microbiol 8, 361–371.

Raquin, V., Lambrechts, L., 2017. Dengue virus replicates and accumulates in *Aedes aegypti* salivary glands. Virology 507, 75–81.

Ratnasingham, S., Hebert, P.D.N., 2007. BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). Mol Ecol Notes 7, 355–364.

Reeves, L.E., Gillett-Kaufman, J.L., Kawahara, A.Y., Kaufman, P.E., 2018. Barcoding blood meals: New vertebrate-specific primer sets for assigning taxonomic identities to host DNA from mosquito blood meals. PLoS Negl Trop Dis 12, e0006767.

Reinert, J.F., 1973. Contributions to the mosquito fauna of Southeast Asia - XVI. Genus *Aedes* Meigen, subgenus Aedimorphus Theobald in Southeast Asia. Contrib Am Entomol Inst 9, 1–218.

Reinhold, J., Lazzari, C., Lahondère, C., 2018. Effects of the Environmental Temperature on *Aedes aegypti* and *Aedes albopictus* Mosquitoes: A Review. Insects 9, 158.

Reisen, W.K., Boyce, K., Cummings, R.C., Delgado, O., Gutierrez, A., Meyer, R.P., Scott, T.W., 1999. Comparative effectiveness of three adult mosquito sampling methods in habitats representative of four different biomes of California. J Am Mosq Control Assoc 15, 24–31.

Rezza, G., Nicoletti, L., Angelini, R., Romi, R., Finarelli, A., Panning, M., Cordioli, P., Fortuna, C., Boros, S., Magurano, F., Silvi, G., Angelini, P., Dottori, M., Ciufolini, M., Majori, G., Cassone, A., 2007. Infection with chikungunya virus in Italy: an outbreak in a temperate region. Lancet 370, 1840–1846.

Rhodes, C.G., Scavo, N.A., Finney, M., Fimbres-Macias, J.P., Lively, M.T., Strauss, B.H., Hamer, G.L., 2022. Meta-Analysis of the Relative Abundance of Nuisance and Vector Mosquitoes in Urban and Blue-Green Spaces. Insects 13, 271.

Ribeiro, M., Antunes, C.M. de F., 2009. Febre amarela: estudo de um surto. Rev Soc Bras Med Trop 42, 523–531.

Riccardo, F., Venturi, G., Di Luca, M., Del Manso, M., Severini, F., Andrianou, X., Fortuna, C., Remoli, M.E., Benedetti, E., Caporali, M.G., Fratto, F., Mignuoli, A.D., Rizzo, L., De Vito, G., De Giorgio, V., Surace, L., Vairo, F., Angelini, P., Re, M.C., Amendola, A., Fiorentini, C., Marsili, G., Toma, L., Boccolini, D., Romi, R., Pezzotti, P., Rezza, G., Rizzo, C., 2019. Secondary Autochthonous Outbreak of Chikungunya, Southern Italy, 2017. Emerg Infect Dis 25, 2093–2095.

Rizzoli, A., Bolzoni, L., Chadwick, E.A., Capelli, G., Montarsi, F., Grisenti, M., de la Puente, J.M., Muñoz, J., Figuerola, J., Soriguer, R., Anfora, G., Di Luca, M., Rosà, R., 2015. Understanding West Nile virus ecology in Europe: Culex pipiens host feeding preference in a hotspot of virus emergence. Parasit Vectors 8, 213.

Robert, V., Günay, F., Goff, G. Le, Boussès, P., Sulesco, T., Khalin, A., Medlock, J.M., Kampen, H., Petrić, D., Schaffner, F., 2019. Distribution chart for Euro-Mediterranean mosquitoes (western Palaearctic region). J Eur Mosq Control Assoc 37, 1–28.

Robinson, A., Busula, A.O., Voets, M.A., Beshir, K.B., Caulfield, J.C., Powers, S.J., Verhulst, N.O., Winskill, P., Muwanguzi, J., Birkett, M.A., Smallegange, R.C., Masiga, D.K., Mukabana, W.R., Sauerwein, R.W., Sutherland, C.J., Bousema, T., Pickett, J.A., Takken, W., Logan, J.G., de Boer, J.G., 2018. *Plasmodium* -associated changes in human odor attract mosquitoes. Proc Natl Acad Sci 115, E4209–E4218.

Roche, B., Léger, L., L'Ambert, G., Lacour, G., Foussadier, R., Besnard, G., Barré-Cardi, H., Simard, F., Fontenille, D., 2015. The Spread of *Aedes albopictus* in Metropolitan France: Contribution of Environmental Drivers and Human Activities and Predictions for a Near Future. PLoS One 10, e0125600.

Roiz, D., Eritja, R., Escosa, R., Lucientes, J., Marquès, E., Melero-Alcíbar, R., Ruiz, S., Molina, R., 2007. A survey of mosquitoes breeding in used tires in Spain for the detection of imported potential vector species. J Vector Ecol 32, 10–15.

Roiz, D., Ruiz, S., Soriguer, R., Figuerola, J., 2015. Landscape Effects on the Presence, Abundance and Diversity of Mosquitoes in Mediterranean Wetlands. PLoS One 10, e0128112.

Roiz, D., Ruiz, S., Soriguer, R., Figuerola, J., 2014. Climatic effects on mosquito abundance in Mediterranean wetlands. Parasit Vectors 7, 333.

Roiz, D., Vazquez, A., Rosà, R., Muñoz, J., Arnoldi, D., Rosso, F., Figuerola, J., Tenorio, A., Rizzoli, A., 2012. Blood meal analysis, flavivirus screening, and influence of meteorological variables on the dynamics of potential mosquito vectors of West Nile virus in northern Italy. J Vector Ecol 37, 20–28.

Romanowski, M., Candeletti, T.M., 1984. Identification and surveillance of *Coquillettidia perturbans* breeding habitat, with observations on larviciding techniques, in Ocean County. Ocean County, NJ Proc NJ Mosq Control Assoc 71, 54–58.

Romi, R., Sabatinelli, G., Savelli, L.G., Raris, M., Zago, M., Malatesta, R., 1997. Identification of a North American mosquito species, *Aedes atropalpus* (Diptera: Culicidae), in Italy. J Am Mosq Control Assoc 13, 245–246.

Rose, N.H., Sylla, M., Badolo, A., Lutomiah, J., Ayala, D., Aribodor, O.B., Ibe, N., Akorli, J., Otoo, S., Mutebi, J.-P., Kriete, A.L., Ewing, E.G., Sang, R., Gloria-Soria, A., Powell, J.R., Baker, R.E., White, B.J., Crawford, J.E., McBride, C.S., 2020. Climate and Urbanization Drive Mosquito Preference for Humans. Curr Biol 30, 3570–3579.

Ruairuen, W., Amnakmanee, K., Primprao, O., Boonrod, T., 2022. Effect of ecological factors and breeding habitat types on Culicine larvae occurrence and abundance in residential areas Southern Thailand. Acta Trop 234, 106630.

Rubio-Palis, Y., Moreno, J.E., Sánchez, V., Estrada, Y., Anaya, W., Bevilacqua, M., Cárdenas, L., Martínez, Á., Medina, D., 2012. Can Mosquito Magnet® substitute for human-landing catches to sample anopheline populations? Mem Inst Oswaldo Cruz 107, 546–549.

Rudnick, A., 1965. Studies of the Ecology of Dengue in Malaysia: A Preliminary Report. J Med Entomol 2, 203–208.

Rudolf, M., Czajka, C., Börstler, J., Melaun, C., Jöst, H., von Thien, H., Badusche, M., Becker, N., Schmidt-Chanasit, J., Krüger, A., Tannich, E., Becker, S., 2013. First Nationwide Surveillance of *Culex pipiens* Complex and *Culex torrentium* Mosquitoes Demonstrated the Presence of *Culex pipiens* Biotype *pipiens/molestus* Hybrids in Germany. PLoS One 8, e71832.

Rueda, J., Hernández, R., Benavent, J.M., Benedito-Durà, V., 2017. Contribución al conocimiento de los mosquitos (Diptera, Culicidae) de la Devesa y del Racó de l’Olla, Parque Natural de l’Albufera de Valencia (España). An Biol 39, 63–73.

Rueda, J., Hernández, R., Benavent, J.M., Sacco, M., Benedito-Durà, V., 2020. Primera cita de *Coquillettidia (Coquillettidia) richiardii* Ficalbi, 1899 (Diptera: Culicidae) para la Comunidad Valenciana. An Biol 37, 129–132.

Ruiz-Arrondo, I., Alarcón-Elbal, P.M., Blanco-Sierra, L., Delacour-Estrella, S., de Blas, I., Oteo, J.A., 2023. Species Composition and Population Dynamics of Culicidae during their Peak Abundance Period in Three Peri-Urban Aquatic Ecosystems in Northern Spain. Diversity 15, 938.

Ruiz-Arrondo, I., Delacour, S., Alarcón-Elbal, P., Pinal, R., Muñoz, A., Bengoa, M., Castillo, J.A., Lucientes, J., 2010. Confirmación de la presencia de *Ochlerotatus* (*Ochlerotatus*) *sticticus* (Meigen, 1838) (Diptera: Culicidae) en la Península Ibérica. Segunda cita de España y primera de Aragón. Bol Soc Entomol Aragonesa 46, 428.

Ruiz-Arrondo, I., Hernández-Triana, L.M., Nikolova, N.I., Fooks, A.R., Oteo, J.A., 2020. Integrated Approaches in Support of Taxonomic Identification of Mosquitoes (Diptera: Culicidae) in Vector Surveillance in Spain. Vector-Borne Zoonotic Dis 20, 831–842.

Ruiz-Arrondo, I., Hernández-Triana, L.M., Oteo-Revuelta, J.A., 2017. Fauna de mosquitos (Diptera, Culicidae) presentes en el humedal de La Grajera (Logroño) y sus implicaciones en salud pública. Zubia 35, 123–140.

Ruiz-Arrondo, I., McMahon, B.J., Hernández-Triana, L.M., Santibañez, P., Portillo, A., Oteo, J.A., 2019. Surveillance of Mosquitoes (Diptera, Culicidae) in a Northern Central Region of Spain: Implications for the Medical Community. Front Vet Sci 6, 86.

Ryan, S.J., Carlson, C.J., Mordecai, E.A., Johnson, L.R., 2019. Global expansion and redistribution of *Aedes*-borne virus transmission risk with climate change. PLoS Negl Trop Dis 13, e0007213.

Rydzanicz, K., Czułowska, A., Dyczko, D., Kiewra, D., 2021. Assessment of mosquito larvae (Diptera: Culicidae) productivity in urban cemeteries in Wroclaw (SW Poland). Int J Trop Insect Sci 41, 3249–3255.

Samanidou-Voyadjoglou, A., Harbach, R.E., 2001. Keys to the adult female mosquitoes (Culicidae) of Greece. Eur Mosq Bull 10, 13–20.

Santa-Ana, M., Khadem, M., Capela, R., 2006. Natural Infection of *Culex theileri* (Diptera: Culicidae) with *Dirofilaria immitis* (Nematoda: Filarioidea) on Madeira Island, Portugal. J Med Entomol 43, 104–106.

Santa-Olalla Peralta, P., Vazquez-Torres, M.C., Latorre-Fandós, E., Mairal-Claver, P., Cortina-Solano, P., Puy-Azón, A., Adiego Sancho, B., Leitmeyer, K., Lucientes-Curdi, J., Sierra-Moros, M.J., 2010. First autochthonous malaria case due to *Plasmodium vivax* since eradication, Spain, October 2010. Euro Surveill 15, 19684.

Sáringer-Kenyeres, M., Bauer, N., Kenyeres, Z., 2020. Active dispersion, habitat requirements and human biting behaviour of the invasive mosquito *Aedes japonicus japonicus* (Theobald, 1901) in Hungary. Parasitol Res 119, 403–410.

Sáringer-Kenyeres, M., Tóth, S., Kenyeres, Z., 2018. Updated checklist of the mosquitoes (Diptera: Culicidae) of Hungary. J Eur Mosq Control Assoc 36, 14–16.

Sauer, F.G., Grave, J., Lühken, R., Kiel, E., 2021. Habitat and microclimate affect the resting site selection of mosquitoes. Med Vet Entomol 35, 379–388.

Schäfer, M., Storch, V., Kaiser, A., Beck, M., Becker, N., 1997. Dispersal behavior of adult snow melt mosquitoes in the Upper Rhine Valley, Germany. J Vector Ecol 22, 1–5.

Schäfer, M.L., Lundström, J.O., 2009. The Present Distribution and Predicted Geographic Expansion of the Floodwater Mosquito *Aedes sticticus* in Sweden. J Vector Ecol 34, 141–147.

Schäfer, M.L., Lundström, J.O., 2006. Different responses of two floodwater mosquito species, *Aedes vexans* and *Ochlerotatus sticticus* (Diptera: Culicidae), to larval habitat drying. J Vector Ecol 31, 123–128.

Schaffner, F., Angel, G., Geoffroy, B., Hervy, J.P., Rhaiem, A., Brunhes, J., 2001. The Mosquitoes of Europe, an Identification and Training Programme CD-ROM.

Schaffner, F., Chouin, S., Guilloteau, J., 2003. First record of *Ochlerotatus* (Finlaya) *japonicus japonicus* (Theobald, 1901) in metropolitan France. J Am Mosq Control Assoc 19, 1–5.

Schaffner, F., Gatt, P., Mall, S., Maroli, M., Spiteri, G., Melillo, T., Zeller, H., 2010. Mosquitoes in Malta: Preliminary entomological investigation and risk assessment for vector-borne diseases (Diptera: Culicidae). Bull Entomol Soc Malta 3, 41–54.

Schaffner, F., Kaufmann, C., Hegglin, D., Mathis, A., 2009. The invasive mosquito *Aedes japonicus* in Central Europe. Med Vet Entomol 23, 448–451.

Schaffner, F., Medlock, J.M., Bortel, W. Van, 2013. Public health significance of invasive mosquitoes in Europe. Clin. Microbiol Infect 19, 685–692.

Schaffner, F., Thiéry, I., Kaufmann, C., Zettor, A., Lengeler, C., Mathis, A., Bourgouin, C., 2012. *Anopheles plumbeus* (Diptera: Culicidae) in Europe: a mere nuisance mosquito or potential malaria vector? Malar J 11, 393.

Schoener, E., Uebleis, S.S., Cuk, C., Nawratil, M., Obwaller, A.G., Zechmeister, T., Lebl, K., Rádrová, J., Zittra, C., Votýpka, J., Fuehrer, H.-P., 2018. Trypanosomatid parasites in Austrian mosquitoes. PLoS One 13, e0196052.

Scholte, E.J., Den Hartog, W., Braks, M., Reusken, C., Dik, M., Hessels, A., 2009. First report of a North American invasive mosquito species *Ochlerotatus atropalpus* (Coquillett) in the Netherlands, 2009. Euro Surveill 14, 19400.

Scholte, E.J., Den Hartog, W., Dik, M., Schoelitsz, B., Brooks, M., Schaffner, F., Foussadier, R., Braks, M., Beeuwkes, J., 2010. Introduction and control of three invasive mosquito species in the Netherlands, July-October 2010. Euro Surveill 15, 19710.

Scholte, E.-J., Jacobs, F., Linton, Y.-M., Dijkstra, E., Fransen, J., Takken, W., 2007. First Record of *Aedes* (*Stegomyia*) *albopictus* in the Netherlands. J Eur Mosq Control Assoc 22, 5–9.

Scholte, E.-J., Schaffner, F., 2008. 14. Waiting for the tiger: establishment and spread of the *Aedes albopictus* mosquito in Europe, in: Emerging Pests and Vector-Borne Diseases in Europe. pp. 241–260.

Schönenberger, A.C., Wagner, S., Tuten, H.C., Schaffner, F., Torgerson, P., Furrer, S., Mathis, A., Silaghi, C., 2016. Host preferences in host-seeking and blood-fed mosquitoes in Switzerland. Med Vet Entomol 30, 39–52.

Seidel, B., Nowotny, N., Duh, D., Indra, A., Hufnagl, P., Allerberger, F., 2013. First records of the thermophilic mosquito *Culiseta longiareolata* (Macquart, 1838) in Austria, 2012, and in Slovenia, 2013. J Eur Mosq Control Assoc 31, 17–20.

Service, 2012. Medical Entomology for Students, 5th ed. Cambridge University Press, Cambridge.

Service, M.W., 1977. A critical review of procedures for sampling populations of adult mosquitoes. Bull Entomol Res 67, 343–382.

Service, M.W., 1971. Flight periodicities and vertical distribution of *Aedes cantons* (Mg.), *Ae. geniculatus* (Ol.), *Anopheles plumbeus* Steph. and *Culex pipiens* L. (Dipt., Culicidae) in southern England. Bull Entomol Res 60, 639–651.

Severini, F., Toma, L., Di Luca, M., Romi, R., 2009. Le Zanzare Italiane: Generalità e Identificazione Degli Adulti (Diptera: Culicidae). Fragm Entomol 41, 213.

Shaalan, E.A.S., Canyon, D. V., 2009. Aquatic insect predators and mosquito control. Trop Biomed 26, 223–261.

Shaman, J., Day, J.F., 2007. Reproductive Phase Locking of Mosquito Populations in Response to Rainfall Frequency. PLoS One 2, e331.

Shestakov, V.I., Mikheeva, A.L., 1966. Contribution to study of Japanese encephalitis vectors in Primorye region. Med Parazitol 35, 545–550.

Shililu, J., Ghebremeskel, T., Seulu, F., Mengistu, S., Fekadu, H., Zerom, M., Ghebregziabiher, A., Sintasath, D., Bretas, G., Mbogo, C., Githure, J., Brantly, E., Novak, R., Beier, J.C., 2003.

Larval Habitat Diversity and Ecology of Anopheline Larvae in Eritrea. J Med Entomol 40, 921–929.

Silaghi, C., Beck, R., Capelli, G., Montarsi, F., Mathis, A., 2017. Development of *Dirofilaria immitis* and *Dirofilaria repens* in *Aedes japonicus* and *Aedes geniculatus*. Parasit Vectors 10, 94.

Silva, N.I.O., Sacchetto, L., de Rezende, I.M., Trindade, G. de S., LaBeaud, A.D., de Thoisy, B., Drumond, B.P., 2020. Recent sylvatic yellow fever virus transmission in Brazil: the news from an old disease. Virol J 17, 9.

Silver, J.B., 2008. Mosquito Ecology, 3rd Edition. ed, Springer. Springer Netherlands, Dordrecht.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene Sequences and a Compilation of Conserved Polymerase Chain Reaction Primers. Ann Entomol Soc Am 87, 651–701.

Simonin, Y., 2024. Circulation of West Nile Virus and Usutu Virus in Europe: Overview and Challenges. Viruses 16, 599.

Simonsen, P.E., Mwakitalu, M.E., 2013. Urban lymphatic filariasis. Parasitol Res 112, 35–44.

Şimşek, F.M., 2004. Seasonal larval and adult population dynamics and breeding habitat diversity of *Culex theileri* Theobald, 1903 (Diptera: Culicidae) in the Gölbaşi District, Ankara, Turkey. Turkish Journal of Zoology 28, 337–344.

Siraj, A.S., Santos-Vega, M., Bouma, M.J., Yadeta, D., Carrascal, D.R., Pascual, M., 2014. Altitudinal Changes in Malaria Incidence in Highlands of Ethiopia and Colombia. Science 343, 1154–1158.

Smallegange, R.C., Verhulst, N.O., Takken, W., 2011. Sweaty skin: An invitation to bite? Trends Parasitol 27, 143–148.

Smith, J.L., Fonseca, D.M., 2004. Rapid Assays for Identification of Members of the *Culex* (*Culex*) *pipiens* Complex, their Hybrids, and Other Sibling Species (Diptera: Culicidae). Am J Trop Med Hyg 70, 339–345.

Snow, K.R., Medlock, J.M., 2006. The potential impact of climate change on the distribution and prevalence of mosquitoes in Britain. Eur Mosq Bull 21, 1–10.

Somboon, P., Phanitchakun, T., Namgay, R., Harbach, R.E., 2023. Review of the Mimeticus Subgroup of *Culex* (*Culex*) (Diptera: Culicidae), With an Assessment of the Specific Status of Three Nominal Species Described from India. J Med Entomol 60, 255–271.

Soto, A., De Coninck, L., Devlies, A.S., Van De Wiele, C., Rosas, A.L.R., Wang, L., Matthijnssens, J., Delang, L., 2023. Belgian *Culex pipiens pipiens* are competent vectors for West Nile virus while *Culex modestus* are competent vectors for Usutu virus. PLoS Negl Trop Dis 17, e0011649.

Soto, A., Delang, L., 2023. *Culex modestus*: the overlooked mosquito vector. Parasit Vectors 16, 373.

Stanton, A.T., 1920. The Mosquitos of Far Eastern Ports with special reference to the Prevalence of *Stegomyia fasciata*, F Bull Entomol Res 10, 333–344.

Succo, T., Leparc-Goffart, I., Ferré, J.-B., Roiz, D., Broche, B., Maquart, M., Noel, H., Catelinois, O., Entezam, F., Caire, D., Jourdain, F., Esteve-Moussion, I., Cochet, A., Paupy, C., Rousseau, C., Paty, M.-C., Golliot, F., 2016. Autochthonous dengue outbreak in Nîmes, South of France, July to September 2015. Euro Surveill 21, 30240.

Şuleşco, T., von Thien, H., Toderaş, L., Toderaş, I., Lühken, R., Tannich, E., 2016. Circulation of *Dirofilaria repens* and *Dirofilaria immitis* in Moldova. Parasit Vectors 9, 627.

Suter, T., Flacio, E., Fariña, B.F., Engeler, L., Tonolla, M., Müller, P., 2015. First report of the invasive mosquito species *Aedes koreicus* in the Swiss-Italian border region. Parasit Vectors 8, 402.

Swan, T., Russell, T.L., Staunton, K.M., Field, M.A., Ritchie, S.A., Burkot, T.R., 2022. A literature review of dispersal pathways of *Aedes albopictus* across different spatial scales: implications for vector surveillance. Parasit Vectors 15, 303.

Taheri, S., González, M.A., Ruiz-López, M.J., Magallanes, S., Delacour-Estrella, S., Lucientes, J., Bueno-Marí, R., Martínez-de la Puente, J., Bravo-Barriga, D., Frontera, E., Polina, A., Martinez-Barciela, Y., Pereira, J.M., Garrido, J., Aranda, C., Marzal, A., Ruiz-Arrondo, I., Oteo, J.A., Ferraguti, M., Gutíerrez-López, R., Estrada, R., Miranda, M.Á., Barceló, C., Morchón, R., Montalvo, T., Gangoso, L., Goiri, F., García-Pérez, A.L., Ruiz, S., Fernandez-Martinez, B., Gómez-Barroso, D., Figuerola, J., 2024. Modelling the spatial risk of malaria through probability distribution of *Anopheles maculipennis* s.l. and imported cases. Emerg Microbes Infect 13, 2343911.

Tahir, F., Bansal, D., Rehman, A. ur, Ajjur, S.B., Skariah, S., Belhaouari, S.B., Al-Romaihi, H., Al-Thani, M.H.J., Farag, E., Sultan, A.A., Al-Ghamdi, S.G., 2023. Assessing the impact of climate conditions on the distribution of mosquito species in Qatar. Front Public Health 10, 970694.

Takken, W., Verhulst, N.O., 2013. Host Preferences of Blood-Feeding Mosquitoes. Annu Rev Entomol 58, 433–453.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol 30, 2725–2729.

Tanaka, K., Mizusawa, K., Saugstad, E.S., 1979. A revision of the adult and larval mosquitoes of Japan (including the Ryukyu Archipelago and the Ogasawara Islands) and Korea (Diptera: Culicidae). Contrib Ameri Ent Inst 16.

Tempelis, C.H., 1975. Host-Feeding Patterns of Mosquitoes, with a Review of Advances in Analysis of Blood Meals by Serology. J Med Entomol 11, 635–653.

Tiawsirisup, S., Kinley, J.R., Tucker, B.J., Evans, R.B., Rowley, W.A., Platt, K.B., 2008. Vector competence of *Aedes vexans* (Diptera: Culicidae) for West Nile virus and potential as an enzootic vector. J Med Entomol 45, 452–457.

Tolle, M.A., 2009. Mosquito-borne Diseases. Curr Probl Pediatr Adolesc Health Care 39, 97–140.

Toma, T., Miyagi, I., Tamashiro, M., 2014. Blood Meal Identification and Feeding Habits of *Uranotaenia* Species Collected in the Ryukyu Archipelago. J Am Mosq Control Assoc 30, 215–218.

Tomazatos, A., Cadar, D., Török, E., Maranda, I., Horváth, C., Keresztes, L., Spinu, M., Jansen, S., Jöst, H., Schmidt-Chanasit, J., Tannich, E., Lühken, R., 2018. Circulation of *Dirofilaria immitis* and *Dirofilaria repens* in the Danube Delta Biosphere Reserve, Romania. Parasit Vectors 11, 392.

Townroe, S., Callaghan, A., 2014. British Container Breeding Mosquitoes: The Impact of Urbanisation and Climate Change on Community Composition and Phenology. PLoS One 9, e95325.

Turell, M.J., O'Guinn, M.L., Dohm, D.J., Jones, J.W., 2001. Vector Competence of North American Mosquitoes (Diptera: Culicidae) for West Nile Virus. J Med Entomol 38, 130–134.

Uelmen, J.A., Lamcyzk, B., Irwin, P., Bartlett, D., Stone, C., Mackay, A., Arsenault-Benoit, A., Ryan, S.J., Mutebi, J.-P., Hamer, G.L., Fritz, M., Smith, R.L., 2023. Human biting mosquitoes and implications for West Nile virus transmission. Parasit Vectors 16, 2.

United Nations, 2019. World Urbanization Prospects 2018 Highlights [WWW Document]. United Nations. URL https://population.un.org/wup/Publications/Files/WUP2018-Highlights.pdf (accessed 5.1.24).

Valerio, L., Marini, F., Bongiorno, G., Facchinelli, L., Pombi, M., Caputo, B., Maroli, M., della Torre, A., 2010. Host-Feeding Patterns of *Aedes albopictus* (Diptera: Culicidae) in Urban and Rural Contexts within Rome Province, Italy. Vector-Borne Zoonotic Dis 10, 291–294.

Van De Vossenberg, B.T.L.H., Ibáñez-Justicia, A., Metz-Verschure, E., Van Veen, E.J., Bruil-Dieters, M.L., Scholte, E.J., 2015. Real-time PCR Tests in Dutch Exotic Mosquito Surveys; Implementation of *Aedes aegypti* and *Aedes albopictus* Identification Tests, and the Development of Tests for the Identification of *Aedes atropalpus* and *Aedes japonicus japonicus* (Diptera: Culicidae). J Med Entomol 52, 336–350.

Van den Eynde, C., Sohier, C., Matthijs, S., De Regge, N., 2023. Belgian *Anopheles plumbeus* Mosquitoes Are Competent for Japanese Encephalitis Virus and Readily Feed on Pigs, Suggesting a High Vectorial Capacity. Microorganisms 11, 1386.

van Riper, C., van Riper, S.G., Goff, M.L., Laird, M., 1986. The Epizootiology and Ecological Significance of Malaria in Hawaiian Land Birds. Ecol Monogr 56, 327–344.

Venables, W.N., Ripley, B.D., 2002. Modern applied statistics with S, Fourth. ed, Statistics and computing. Springer, New York, NY.

Venturi, G., Di Luca, M., Fortuna, C., Remoli, M.E., Riccardo, F., Severini, F., Toma, L., Del Manso, M., Benedetti, E., Caporali, M.G., Amendola, A., Fiorentini, C., De Liberato, C., Giammattei, R., Romi, R., Pezzotti, P., Rezza, G., Rizzo, C., 2017. Detection of a chikungunya outbreak in central Italy, August to September 2017. Euro Surveill 22, 17–00646.

Veronesi, R., Gentile, G., Carrieri, M., Maccagnani, B., Stermieri, L., Bellini, R., 2012. Seasonal pattern of daily activity of *Aedes caspius*, *Aedes detritus*, *Culex modestus*, and *Culex pipiens* in the Po Delta of northern Italy and significance for vector-borne disease risk assessment. J Vector Ecol 37, 49–61.

Versteirt, V., Pecor, J., Fonseca, D.M., Coosemans, M., Bortel, W. Van, 2014. Confirmation of *Aedes koreicus* (Diptera: Culicidae) in Belgium and description of morphological differences between Korean and Belgian specimens validated by molecular identification. Zootaxa 3191, 21–32.

Versteirt, V., Schaffner, F., Garros, C., Dekoninck, W., Coosemans, M., Van Bortel, W., 2009. Introduction and Establishment of the Exotic Mosquito Species *Aedes japonicus japonicus* (Diptera: Culicidae) in Belgium. J Med Entomol 46, 1464–1467.

Vezzani, D., 2007. Review: Artificial container-breeding mosquitoes and cemeteries: a perfect match. Trop Med Int Heal 12, 299–313.

Vicente, J.L., Sousa, C.A., Alten, B., Caglar, S.S., Falcutá, E., Latorre, J.M., Toty, C., Barré, H., Demirci, B., Di Luca, M., Toma, L., Alves, R., Salgueiro, P., Silva, T.L., Bargues, M.D., Mas-Coma, S., Boccolini, D., Romi, R., Nicolescu, G., do Rosário, V.E., Ozer, N., Fontenille, D., Pinto, J., 2011. Genetic and phenotypic variation of the malaria vector *Anopheles atroparvus* in southern Europe. Malar J 10, 5.

Vilibic-Cavlek, T., Savic, V., Petrovic, T., Toplak, I., Barbic, L., Petric, D., Tabain, I., Hrnjakovic-Cvjetkovic, I., Bogdanic, M., Klobucar, A., Mrzljak, A., Stevanovic, V., Dinjar-Kujundzic, P., Radmanic, L., Monaco, F., Listes, E., Savini, G., 2019. Emerging Trends in the Epidemiology of West Nile and Usutu Virus Infections in Southern Europe. Front Vet Sci 6, 437.

Vinogradova, E.B., 2000. *Culex pipiens pipiens* mosquitoes: taxonomy, distribution, ecology, physiology, genetics, applied importance and control. Pensoft Publishers, Moscow.

Vogels, C.B., Göertz, G.P., Pijlman, G.P., Koenraadt, C.J., 2017. Vector competence of European mosquitoes for West Nile virus. Emerg Microbes Infect 6, 1–13.

Vogels, C.B.F., Hartemink, N., Koenraadt, C.J.M., 2017. Modelling West Nile virus transmission risk in Europe: effect of temperature and mosquito biotypes on the basic reproduction number. Sci Rep 7, 5022.

Vogels, C.B.F., Van De Peppel, L.J.J., Van Vliet, A.J.H., Westenberg, M., Ibañez-Justicia, A., Stroo, A., Buijs, J.A., Visser, T.M., Koenraadt, C.J.M., 2015. Winter activity and aboveground hybridization between the two biotypes of the West Nile Virus vector *Culex pipiens*. Vector-Borne Zoonotic Dis 15, 619–626.

Wagner, S., Mathis, A., Schönenberger, A.C., Becker, S., Schmidt-Chanasit, J., Silaghi, C., Veronesi, E., 2018. Vector competence of field populations of the mosquito species *Aedes japonicus japonicus* and *Culex pipiens* from Switzerland for two West Nile virus strains. Med Vet Entomol 32, 121–124.

Wang, L., Rosales Rosas, A.L., De Coninck, L., Shi, C., Bouckaert, J., Matthijnssens, J., Delang, L., 2021. Establishment of *Culex modestus* in Belgium and a Glance into the Virome of Belgian Mosquito Species. mSphere 6, e01229-20.

Wasserman, S., Tambyah, P.A., Lim, P.L., 2016. Yellow fever cases in Asia: primed for an epidemic. Int J Infect Dis 48, 98–103.

Weaver, S.C., Charlier, C., Vasilakis, N., Lecuit, M., 2018. Zika, Chikungunya, and Other Emerging Vector-Borne Viral Diseases. Annu Rev Med 69, 395–408.

Weaver, S.C., Reisen, W.K., 2010. Present and future arboviral threats. Antiviral Res 85, 328–345.

Weitzel, T., Braun, K., Collado, A., Jost, A., Becker, N., 2011. Distribution and frequency of *Culex pipiens* and *Culex torrentium* (Culicidae) in Europe and diagnostic allozyme markers. Eur Mosq Bull 29, 22–37.

Weitzel, T., Collado, A., Jöst, A., Pietsch, K., Storch, V., Becker, N., 2009. Genetic Differentiation of Populations within the *Culex pipiens* Complex and Phylogeny of Related Species. J Am Mosq Control Assoc 25, 6–17.

Wellborn, G.A., Skelly, D.K., Werner, E.E., 1996. Mechanisms Creating Community Structure Across a Freshwater Habitat Gradient. Annu Rev Ecol Syst 27, 337–363.

Werner, D., Kronefeld, M., Schaffner, F., Kampen, H., 2012. Two invasive mosquito species, *Aedes albopictus* and *Aedes japonicus japonicus*, trapped in south-west Germany, July to August 2011. Euro Surveill 17, 20067.

Werner, D., Zielke, D.E., Kampen, H., 2016. First record of *Aedes koreicus* (Diptera: Culicidae) in Germany. Parasitol Res 115, 1331–1334.

WHO, 2022a. Dengue and severe dengue [WWW Document]. WHO. URL https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue (accessed 5.1.24).

WHO, 2022b. Lymphatic filariasis [WWW Document]. WHO. URL https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis (accessed 5.1.24).

WHO, 2021. World Malaria Report 2021 [WWW Document]. URL https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2021 (accessed 5.1.24).

WHO, 2020a. Vector-borne diseases [WWW Document]. WHO. URL https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases (accessed 5.1.24).

WHO, 2020b. Chikungunya [WWW Document]. WHO. URL https://www.who.int/news-room/fact-sheets/detail/chikungunya (accessed 5.1.24).

WHO, 2019. Yellow Fever [WWW Document]. URL https://www.who.int/news-room/fact-sheets/detail/yellow-fever (accessed 5.1.24).

WHO, 2018. Zika Virus [WWW Document]. URL https://www.who.int/news-room/fact-sheets/detail/zika-virus (accessed 5.1.24).

Wilke, André B.B., Benelli, G., Beier, J.C., 2021. Anthropogenic changes and associated impacts on vector-borne diseases. Trends Parasitol 37, 1027–1030.

Wilke, A.B.B., Chase, C., Vasquez, C., Carvajal, A., Medina, J., Petrie, W.D., Beier, J.C., 2019. Urbanization creates diverse aquatic habitats for immature mosquitoes in urban areas. Sci Rep 9, 15335.

Wilke, A.B.B., Medeiros-Sousa, A.R., Ceretti-Junior, W., Marrelli, M.T., 2017. Mosquito populations dynamics associated with climate variations. Acta Trop 166, 343–350.

Wilke, André Barretto Bruno, Vasquez, C., Carvajal, A., Medina, J., Chase, C., Cardenas, G., Mutebi, J.-P., Petrie, W.D., Beier, J.C., 2020. Proliferation of *Aedes aegypti* in urban environments mediated by the availability of key aquatic habitats. Sci Rep 10, 12925.

Wilke, André B. B., Vasquez, C., Carvajal, A., Moreno, M., Diaz, Y., Belledent, T., Gibson, L., Petrie, W.D., Fuller, D.O., Beier, J.C., 2020. Cemeteries in Miami-Dade County, Florida are important areas to be targeted in mosquito management and control efforts. PLoS One 15, e0230748.

Wilke, André B. B., Vasquez, C., Carvajal, A., Moreno, M., Fuller, D.O., Cardenas, G., Petrie, W.D., Beier, J.C., 2021. Urbanization favors the proliferation of *Aedes aegypti* and *Culex quinquefasciatus* in urban areas of Miami-Dade County, Florida. Sci Rep 11, 22989.

Wilke, A.B.B., Vasquez, C., Carvajal, A., Moreno, M., Petrie, W.D., Beier, J.C., 2022. Evaluation of the effectiveness of BG-Sentinel and CDC light traps in assessing the abundance, richness, and community composition of mosquitoes in rural and natural areas. Parasit Vectors 15, 51.

Wilkerson, R., Linton, Y., Strickman, D., 2021. Mosquitoes of the World. Johns Hopkins University Press, Baltimore, MD, USA.

Wilkerson, R.C., Linton, Y.-M., Fonseca, D.M., Schultz, T.R., Price, D.C., Strickman, D.A., 2015. Making Mosquito Taxonomy Useful: A Stable Classification of Tribe Aedini that Balances Utility with Current Knowledge of Evolutionary Relationships. PLoS One 10, e0133602.

Wint, W., Jones, P., Kraemer, M., Alexander, N., Schaffner, F., 2022. Past, present and future distribution of the yellow fever mosquito *Aedes aegypti*: The European paradox. Sci Total Environ 847, 157566.

Yan, J., Gangoso, L., Ruiz, S., Soriguer, R., Figuerola, J., Martínez-de la Puente, J., 2021. Understanding host utilization by mosquitoes: determinants, challenges and future directions. Biol Rev 96, 1367–1385.

Yates, M.G., 1979. The biology of the tree-hole breeding mosquito *Aedes geniculatus* (Olivier) (Diptera: Culicidae) in southern England. Bull Entomol Res 69, 611–628.

Yee, D., 2008. Tires as habitats for mosquitoes: A review of studies within the eastern United States. J Med Entomol 45, 581–593.

Young, M.W., Kay, S.A., 2001. Time zones: a comparative genetics of circadian clocks. Nat Rev Genet 2, 702–715.

Zeller, H., Marrama, L., Sudre, B., Bortel, W. Van, Warns-Petit, E., 2013. Mosquito-borne disease surveillance by the European Centre for Disease Prevention and Control. Clin Microbiol Infect 19, 693–698.

Zhang, H., Zhu, Y., Liu, Z., Peng, Y., Peng, W., Tong, L., Wang, J., Liu, Q., Wang, P., Cheng, G., 2022. A volatile from the skin microbiota of flavivirus-infected hosts promotes mosquito attractiveness. Cell 185, 2510-2522.e16.

Zhao, J., Tang, T., Wang, X., 2020. Effects of landscape composition on mosquito population in urban green spaces. Urban For Urban Green 49, 126626.

Zhou, Y.-H., Zhang, Z.-W., Fu, Y.-F., Zhang, G.-C., Yuan, S., 2018. Carbon Dioxide, Odorants, Heat and Visible Cues Affect Wild Mosquito Landing in Open Spaces. Front Behav Neurosci 12, 86.

Zittra, C., Waringer, J., Werblow, A., Melaun, C., Fuehrer, H.-P., 2014. Reconfirmation of *Culiseta* (Allotheobaldia) *longiareolata* (Macquart 1838) (Diptera: Culicidae) in Austria. The first sequence-confirmed findings in northeastern Austria. Acta Zoobot Austria 150/151, 17–24.

VIII. Academic and research activities of the author

ACADEMIC RECORD

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PUBLICATIONS

Taheri, S., González, M. A., Ruiz-López, M. J., Magallanes, S., Delacour-Estrella, S., …. **Goiri, F**…….. & Figuerola, J. (2024). Modeling the spatial risk of malaria through probability distribution of *Anopheles maculipennis* sl and imported cases. *Emerging Microbes & Infections,* 13(1), 2343911. https://doi.org/10.1080/22221751.2024.2343911

Goiri, F., González, M. A., Cevidanes, A., Barandika, J. F., & García-Peréz, A. L. (2024). Mosquitoes in urban green spaces and cemeteries in northern Spain. *Parasites & Vectors*, *17*(1), 168. https://doi.org/10.1186/s13071-024-06263-z

Cevidanes, A., **Goiri, F.**, Barandika, J.F., Vázquez, P., Goikolea, J., Zuazo, A., Etxarri, N., Ocio, G., García-Pérez, A.L. (2023) "Invasive *Aedes* mosquitoes in an urban - peri-urban gradient in northern Spain: Evidence of the wide distribution of *Aedes japonicus*". *Parasites & Vectors,* 16(1), 234. https://doi.org/10.1186/s13071-023-05862-6

González, M.A.*, **Goiri, F*,** Cevidanes A, Hernández-Triana, L.M, Barandika, J.F., García-Pérez, A.L. (2023) "Mosquito community composition in two major stopover aquatic ecosystems in northern Spain". *Medical and Veterinary Entomology*, 37(3), 616-629. *EQUAL CONTRIBUTION. https://doi.org/10.1111/mve.12661

Miranda M.A., Barceló, C., Arnoldi, D., Augsten, X., …..**Goiri, F.**, …… della Torre, A.. (2022) "AIMSurv: First pan-European harmonized surveillance of Aedes invasive mosquito species of relevance for human vector-borne diseases". *Gigabyte*, 2022, 1-13. https://doi.org/10.46471/gigabyte.57

González, M.A, **Goiri, F.,** Prosser, SWF, Cevidanes, A., Hernández-Triana, L.M., Barandika, J.F., Hebert, P.D.N, García-Pérez, A.L (2022) "Culicoides species community composition and feeding preferences in two aquatic ecosystems in Northern Spain". *Parasites & Vectors*, 15 (1), 199. https://doi.org/10.1186/s13071-022-05297-5

González, M. A., Cevidanes, A., **Goiri, F.**, Barandika, J. F., & García-Pérez, A. L. (2021). "Diversity and distribution of larval habitats of mosquitoes (Diptera: Culicidae) in northern Spain: from urban to natural areas". *Journal of Vector Ecology*, 46(2), 173-185. https://doi.org/10.52707/1081-1710-46.2.173

Alarcón-Elbal, P. M., González, M. A., Delacour-Estrella, S., Bravo-Barriga, D., Estrada Peña, R., **Goiri, F.**, García- Pérez, A.L., Lucientes, J. (2021) "First Findings and Molecular Data of *Phlebotomus mascittii* (Diptera: Psychodidae) in the Cantabrian Cornice (Northern Spain)". *Journal of Medical Entomology*, 58(6), 2499–2503. https://doi.org/10.1093/jme/tjab091

González, M. A., Prosser, S. W., Hernández-Triana, L. M., Alarcón-Elbal, P. M., **Goiri, F.**, López, S, Ruiz-Arrondo, I., Hebert, P.D.N., García-Pérez, A.L. (2020) "Avian feeding preferences of *Culex pipiens* and *Culiseta* spp. along an urban-to-wild gradient in northern Spain", *Frontiers in Ecology and Evolution*,8, 568835. https://doi.org/10.3389/fevo.2020.568835

González, M.A; **Goiri, F**; Barandika, J.F; García-Pérez, A.L (2020) "Culicoides biting midges and mosquito fauna at three dog and cat shelters in rural and periurban areas in Northern Spain", *Medical and Veterinary Entomology*, *35*(1), 79-87 https://doi.org/10.1111/mve.12471

Goiri, F; González, M.A.; Goikolea, J.; Oribe, M.; Castro, V.; Delacour, S.; Lucientes, J.; Ortega-Araiztegi, I.; Barandika, J.F.; García-Pérez, A.L. (2020) "Progressive Invasion of *Aedes albopictus* in Northern Spain in The Period 2013–2018 and A Possible Association with the Increase in Insect Bites", *International Journal of Environmental Research and Public Health,* 17, 1678. https://doi.org/10.3390/ijerph17051678

de Tomas, I., Maiz, E., **Goiri, F.**, Yu, K., Toran-Pereg, P., Castrillo, P., & Etxeberria, U. (2020). Mindful eating: Effects of a brief induction in the choice and intake of food in children. *Current Psychology*, 1-11. https://doi.org/10.1007/s12144-020-00764-7

CONTRIBUTION TO CONFERENCES

González M.A., **Goiri F.**, Prosser, S.W., Hernández-Triana, L.M., Hebert, P.D.M., Goikolea, J., Etxarri, N., Aldamiz-Echevarria, R., Ocio, G., Barandika, J.F., Cevidanes, A., García-Pérez, A.L.. Revealing the diversity of blood-sucking Dipteran pests in urban areas of northern Spain. *10th International Conference on Urban Pests*, Barcelona (Spain), June 2022. ORAL COMMUNICATION

Goiri F, Cevidanes, A, Vázquez, P., Barandika, J.F., García-Pérez, A.L., Goikolea, J., and Invasive Mosquito Working group in the Basque Country. Distribution of *Aedes albopictus* and *Aedes japonicus* in urban and peri-urban environments in the Basque Country (northern Spain). *10th International Conference on Urban Pests*, Barcelona (Spain), June 2022. ORAL COMMUNICATION

Goiri, F.; Barandika, J.F.; González, M.A.; Goikolea, J.; Lucientes, J; García-Pérez, A.L. Results on the Surveillance of *Aedes albopictus* in Northern Spain (Basque Country) in the period 2013-2019. *2nd AIM-COST Annual Conference*. Lisbon (Portugal), February 2020. ORAL COMMUNICATION

González, M.A., **Goiri, F.**, Barandika, J.F., García-Pérez, A.L.. First Studies of Culicids in Two Aquatic Ecosystems of International Relevance from Northern Spain. *I Encuentro Internacional de Organismos Vectores Implicados en Transmisión de Entidades Zoonóticas*, Trinidad, (Cuba), November 2019. POSTER

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Six months training (March 2016 – September 2016) at the Department of Molecular Biology and Genetics in the research group of Plant Molecular Biology (Aarhus University, Denmark) under the supervision of Dr. Niels Sandal. Training on: “Importance of LjNFR5 interactors and LjCBS1 in symbiosis with *Mesorizhobium loti*”

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