Journal of the European Mosquito Control Association

ISSN 2054-930X – 2023 Supplement 1 – XI[th] International EMCA Conference

emca
European Mosquito Control Association

Wageningen Academic Publishers

XIth International EMCA Conference

Shaping the future of vector control in Europe

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ISSN 2054-930X online, DOI: 10.52004/JEMCA2023.S1

EMCA 2023 – Forewords and acknowledgements

The EMCA International Conferences aim at discussing outcomes and advances in surveillance and control of mosquitoes, black flies, sand flies and other blood-seeking arthropods.

The abstracts in this JEMCA Supplement is a collation of novel applied research and experimental development presented at the XIth International EMCA Conference (EMCA 2023) entitled 'Shaping the future of vector control in Europe', held in Palma de Mallorca, Spain. With regards to the concern for sustainable pest and vector management, the abstracts cover a wide range of topics addressing mosquitoes and other vectors, with particular emphasis on surveillance and management, the context of disease outbreaks, citizen involvement, challenges in One Health, mosquito control in the Mediterranean region, and innovative technologies and practices in vector control.

A topic that is of great concern to the EMCA is the Best Practices for mosquito control in built-up areas, which is tackled in a round table at the conference, but also as a side event of the relative EMCA Working Group. This WG aims at publishing a Best Practices guidance document by early 2024, as a development of our EMCA Workshop held in 2022 in Mendrisio, Switzerland, and with the support of WHO-TDR-Special Programme for Research and Training in Tropical Diseases.

Together with the conference, a one-day Training Course is organised on the identification of non-target fauna in trappings and mosquito rearing and repellent testing, at the University of the Balearic Islands (UIB) facilities. Finally, an additional side-event is hosted at UIB for WHO, the TDR Workshop on Developing Terms of Reference for Reference Centre's in Medical Entomology Training and Vector Control. This all brings around 200 scientists together for tackling vector concerns.

The EMCA 2023 Scientific Committee is pleased to publish these abstracts in order to disseminate current scientific information to medical and veterinary entomologists, public health and animal health stakeholders, mosquito and other pest control operators, and in this way support evidence-based decisions. It is our goal to promote increased responsiveness towards sustainable vector control. This requires state-of-the art methods within integrated vector management programmes, combining efficacy, cost effectiveness, ecological soundness and sustainability. This will strengthen the support by stakeholders and citizens.

The XIth EMCA International Conference could happen thanks to the effort of all members of the EMCA 2023 Organising Committee, the Scientific Committee and the Training Course Committee, as well as to the EMCA Board members and the EMCA secretary. We are very pleased and grateful to be welcome at the University of the Balearic Islands, with the efficient support of our hosts Dr Carlos Barceló and Prof. Miguel Ángel Miranda. We are grateful to our sponsors who, thanks to their financial support, make the conference affordable to a large audience: Co-Diagnostics, Valent BioSciences, Debug, Igeba Gerätebau, Vectobal, Rentokil Initial, Avia-GIS, Ecodevelopment, Culinex, Entostudio, Icybac, and Martec.

Finally, without attendees the conference would not be successful. Thus we thank all authors of abstracts for their presence and their valued contribution.

Francis Schaffner, PhD
Consultant & Associate Researcher at the University of Zurich, Institute of Parasitology, Switzerland
EMCA 2023 Scientific Committee Chair
EMCA President

Dear attendees

We are very happy and proud to have organised the XI[th] international EMCA conference at the University of the Balearic Islands, Spain. This is the second international meeting organised by our research group after the International SOVE in 2017, consolidating more than 20 years of scientific research activity on the medical and veterinary entomology topic. For this very special occasion, 190 participants involved in vector research and control have registered, offering 117 communications, which is a great success for the biggest EMCA conference ever organised.

Several trends, such as globalisation, climate change and land-use disturbance are favouring the spread of invasive and native vector species in Europe. Mosquito-borne disease outbreaks are increasing year by year; so are disease pathogens transmitted by other very important vector groups such as sand flies, black flies, stable flies and ticks.

The meeting of EMCA at Palma is a great opportunity to share knowledge among professional and researchers involved in vector surveillance and control, as well as to identify future challenges for our community. Please take this opportunity to exchange experiences, learn and discuss with researchers, experts in vector control and students. Increasing networking would also allow to better respond to the increasing demand of holistic, integrated and sustainable strategies for vector control, with regards to the One Health scenario.

We highly appreciate the opportunity given by EMCA to host this meeting and we hope to be live up to the participants expectations from social and scientific point of view.

We want to transmit special thanks to all the members of the Organising, Scientific and Training Course committees for the support provided and the excellent collaboration. Also, to all the sponsors that kindly contributed to financially support the meeting, and the last, but not the least, to all the participants of the conference that are the core of the meeting.

Welcome to Palma!

Yours faithfully,
Dr. Carlos Barceló (Chair) & **Dr. Miguel Ángel Miranda** (Deputy)
ZAP-UIB research group, University of the Balearic Islands, Spain

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Keynote session

ORAL PRESENTATIONS

The isthmus

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Social determinants of health (SDOH) are the nonclinical factors that give rise to 80-90% of an individual's health status. These factors are an amalgamation of the environments in which people are born, raised, employed, and live their lives. These conditions are dynamic and profoundly local in nature. Information ecosystems, while not originally conceived as a SDOH, should be systematically factored into vector intervention programmes reflecting the hyper-localness of the new normal. This condition will require new thinking and information framing by vector and environmental public health professionals. The challenge we encounter is that contemporary vector control practitioners were likely educated on the premise that their major professional functions would be rooted in assessment, assurance, and policy development, with little or no emphasis on communication techniques or combating mis- and disinformation. Regretfully, society has entered the Anthropocene era in which the health, safety and economic prosperity of nations will be increasingly rooted in motivating and activating civil society to contribute to reducing risk at the local level. Fortunately, local agencies are often hotbeds of innovation and as such, represent where action and progress can be achieved. Lessons learned by professionals at the local level can be adopted, amended to reflect local conditions elsewhere and scaled up across jurisdictions. Effective vector-borne disease practitioners and environmental public health thought leaders will recognise how people think, the information ecosystems where they form their opinions, and the role of values, beliefs, and absorbed identities in individual decision-making. Specifically, we will need to accept that falsehoods travel six times faster than the truth, and the acceleration of these rumours occurs where people feel safe. These conditions are mediated in an environment where human relationships with information are emotional. Our role should be to accept and acknowledge that there are often legitimate concerns in falsehoods, that we play a vital role in addressing falsehoods and should offer alternate explanations. Alternately our information should be understandable, engaging, and culturally competent. Additional characteristics of effective communication campaigns include accuracy, accessibility, authenticity and actionability. Aspirational communications, effective implementation of fluency theory, and wise use of words are among the tools environmental public health professionals employ to garner local support for effective vector interventions.

The coordination of the Spanish National Plan for Prevention, Surveillance and Control of Vector-Borne Disease

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The approach to vector-borne diseases (VBD) from a 'One Health' perspective implies the inclusion of objectives and activities from human, animal and environmental health. In 2022-23, a National Plan for the Prevention, Surveillance and Control of VBDs (hereafter 'the Plan') has been drawn up in Spain, focused on disease pathogens transmitted by mosquitoes from genera *Aedes* and *Culex*. During the preparation phase, the Ministry of Health acted as coordinator of the working groups, the structure of the document and the schedules, and presented the document to the public health commission of the inter-territorial council. The working groups worked autonomously, in some cases appointing a group coordinator. The draft was prepared and reviewed by 6 technical groups until reaching a consensus. The risk scenarios for *Aedes*-borne diseases and West Nile fever were agreed upon. Around them, the objectives and activities were established. The entire Plan was then evaluated by the Public Health Commission, until its final approval. The mechanisms for coordinating the Plan once implemented, is structured around Permanent Coordination (PC) and Response Coordination Committees (RCC). The PC is in charge of proposing indicators, evaluating the Plan, ensuring that the information is integrated into the surveillance network, proposing legal regulations and carrying out risk assessments. The RCC is in charge of agreeing on the response and of proposing communication strategies and modifications to the actions defined in the Plan to adapt them to the alert situation. In summary, the Plan from a One Health perspective requires well-structured coordination mechanisms to deal with the changing situations and the numerous actors involved.

In search of new tools towards improving sand fly control strategies

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Sand flies (Diptera, Psychodidae, Phlebotominae) are blood-feeding arthropods of high veterinary and public health importance. They are competent vectors of various pathogens such as *Bartonella* (causative agent of bartonellosis) and *Leishmania* spp. (causative agent of leishmaniasis), as well as arboviruses (such as Phlebovirus, Vesiculovirus and Orbivirus spp.). Leishmaniasis is among the top ten neglected tropical diseases globally infecting approximately one million people every year. According to World Health Organization (WHO) estimations, approximately 350 million people worldwide are at risk of acquiring leishmaniasis, with 20 000–30 000 deaths annually. In an effort to reduce the risk of sand fly-borne diseases, a variety of chemical-based control interventions – i.e. indoors residual spraying, insecticide treated nets, spatial repellents, space sprays – are being applied on a global basis under different contexts, to reduce sand fly populations and the associated biting pressure exerted to affected communities with varied levels of success. The overwhelming majority of these approaches are targeting adult sand flies, mostly at indoor settings, while no methodologies are specifically tailored against sand fly immature stages, due to the current gap in knowledge on the ecology of these stages under field conditions. Current, applied methodologies for sand fly control, in different environmental and cultural contexts are showcased, while operational challenges and gaps in knowledge that may be hindering the successful implementation of sand fly control interventions are elaborated. Future perspectives for improving the efficiency and sustainability of sand fly control programmes, through investment in innovative approaches and tools are discussed.

WHO and Vector Control, from the first recommendations to the current evidence-based guidelines

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In 2023, the World Health Organization (WHO) is celebrating its 75 years and since 1948 WHO is fighting Vector-Borne Diseases (VBDs) through Vector Control (VC) activities, starting with mass campaigns against several VBDs including malaria with spraying of DDT, recommended because of high efficacy. These campaigns have led to the eradication of malaria in many countries, although the environmental impact was not understood. However, after a few years of intensive use, the vectors became resistant to DDT and new insecticides were developed and evaluated against the diseases through improved field trials. Among the new products, successful testing of the Insecticide Treated Nets (ITNs) against malaria with Randomized Control Trials were supported in the mid-1980s. ITNs were consequently recommended, becoming later the program Long-Lasting Insecticide Nets. In 2015, Europe became the first (and still the only) region achieving malaria elimination for all countries. Until recently VC activities were focusing mostly on the insecticides and the WHO Pesticide Evaluation Scheme was active from 1997 to 2022 to provide recommendations. In 2004 it became evident that VC should integrate other approaches such as community participation for the elimination of breeding sites and WHO released the Integrated Vector Management Framework. But, as resistance to insecticide increased and new concerns raised on their environmental impact, WHO moved toward other technologies and approaches and a working group of experts, the Vector Control Advisory Group was created in 2013, to look at the impact of new technologies on the VBDs. The WHO Assessment Team for Prequalification of Vector Control Product (PQT/VCP) was established in 2016 for quality and safety. But the Zika pandemic highlighted the weaknesses of the VC approaches leading to the WHO proposal on Global Vector Control Response for 2017–2030, approved at the World Health Assembly by more than 190 Member States. This Response is based on four pillars which are the strengthening of the multisectoral approaches, the engagement of communities, the enhancement of surveillance and monitoring and the scaling up of innovative tools. The response started to be implemented through the Vector Control Need Assessment for each country. More recently, the WHO guidelines on VC made a step further on improvement of analysis of the evidence and scoring of the recommendations through the Grading of Recommendations approach and more transparency using online tools. The online updated malaria guidelines were released in 2023. WHO activities are under the mandates of the Member States and WHO commitment and capacity to provide the best guidance for vector control will remain strong within the current unpredictable dynamics of VBDs due to the global changes.

Session 1. Surveillance and management of invasive mosquito species

ORAL PRESENTATIONS

The potential use of detection dogs for the surveillance of the Asian tiger mosquito

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The Asian tiger mosquito, *Aedes albopictus*, is one of the most invasive species worldwide and is spreading across Switzerland. It is an aggressive biter and poses a public health threat due to its vector competence for viruses such as dengue, Chikungunya or Zika. A key challenge is early detection of infested areas in order to implement targeted vector control measures promptly. Known for their ability to locate mammals and plants, detection dogs have had promising use in insect location. In the present study we assessed whether detection dogs could potentially be deployed to identify *Ae. albopictus* breeding sites. We trained 6 detection dogs in differentiating odours from *Ae. albopictus*-contaminated water from other odours using an automated training system. The automated training system had 7 holes through which the dogs could sample an odour. In a round, one hole presented the odour of water containing *Ae. albopictus* larvae, while the other 6 holes presented odours from water containing either no larvae or larvae of other mosquito species. The experiment was fully automated and double-blinded; neither the dog guide nor the experimenter knew in which hole the odour from the *Ae. albopictus* larvae was. The training consisted of three blocks of increasing difficulty. Block three comprised an in-lab field simulation using watering cans. Across the 6 dogs, the final success rates were 84.7%, 82.7% and 55.6% for blocks 1, 2 and 3, respectively, while the individual success rates varied consistently between the dogs. We conclude that detection dogs can be trained to identify *Ae. albopictus* larvae within the lab environment but further training needs to be conducted to achieve consistent success in identifying *Ae. albopictus* larvae in the field context.

Introduction and spread of the invasive mosquito *Aedes koreicus* in Italy: insights from population genetics

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The present study focuses on the distribution and population genetics of the invasive Korean bush mosquito, *Aedes koreicus*, which has been spreading in Europe since 2008, likely by human-mediated transportation. The invasiveness success of this alien mosquito is particularly evident in Italy, where the populations of *Ae. koreicus* did not remain confined to relatively small areas, as has instead been observed in other European countries (e.g. Belgium and Germany). Recently, we modelled the distribution and expansion potential of *Ae. koreicus* in Italy, using Species Distribution Modelling (SDM) tools. SDM generated from the occurrence data for *Ae. koreicus* in Italy and Europe predicted further highly suitable areas of colonisation both in our country and in other European countries. Moreover, despite the large number of reports of *Ae. koreicus* in Europe, possible entry routes into the continent have not been reconstructed so far, nor it is clear how the mosquito spread after its first introduction(s). For these reasons, the study of the genetic structure of populations of *Ae. koreicus* and the understanding of the genetic relatedness between individuals from different locations would permit to hypothesise how this mosquito spread in Northern-Italy, and even in other European countries. To achieve this goal, microsatellites markers for the study of the genetic structure of populations of *Ae. koreicus* have been developed. These markers have then been used to investigate different mosquito populations, collected in different localities in the pre-alpine area of Italy and in a neighbouring country (Slovenia). A first analysis on the genetic variation of *Ae. koreicus* populations revealed a high level of relatedness between mosquito individuals present in Italy and in Slovenia; in addition, Bayesian analysis of co-ancestry levels revealed the presence of two main genetic patterns. Demographic analyses are in progress in order to understand if these co-ancestry patterns could be the consequence of distinct introduction events of *Ae. koreicus* in Italy. In prospect, a continuous monitoring of the spread of *Ae. koreicus*, associated with population genetics studies, would allow to discover the geographic origins of European populations of this mosquito, as well as their entrance pathways, which would then permit to prevent or limit further introductions.

Using wooden mouth spatulas as oviposition support in ovitraps: what do we miss?

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Ovitraps are easy-to-use and inexpensive traps for detecting potentially invasive container-breeding *Aedes* mosquitoes, particularly the Asian tiger mosquito (*Aedes albopictus*). These traps consist only of a black container filled with water, and an oviposition support. This oviposition support has, in contrast to the smooth surface of the container, a rough surface, which several *Aedes* mosquitoes prefer for laying their eggs. Commonly used oviposition supports are filter papers, wooden sticks (mansonite or wooden mouth spatula) or polystyrene. Except for filter paper, which covers the entire inner surface of the container, wooden sticks or polystyrene leave the inner surface completely or largely uncovered, which could be used for egg laying as well. The aim of this study is to investigate whether observations of container-breeding *Aedes* species can be missed if only eggs laid on the oviposition support are examined. In 2022, five ovitraps were set up at each of six locations in the province of Salzburg in Austria. Wooden mouth spatulas roughened with sandpaper were used as oviposition support. From the beginning of May until the end of October the traps were checked in weekly intervals. At each check the water and the oviposition support was changed and the inner surface of the container was wiped with a paper tissue. The eggs on the stick and tissue were counted and the species of the eggs determined (using morphological characteristics and multiplex PCR). We found eggs from three *Aedes* species, the alien *Ae. albopictus* and *Ae. japonicus*, as well as the native *Ae. geniculatus*. All of those laid their eggs on the wooden mouth spatula as well as on the container wall. However, the mouth spatula was clearly preferred to the container wall as oviposition substrate. Events where eggs were only found on the container wall were rare. Only in 2.9% (95% CI [1.2%, 5.9%]) of the capture events eggs would have been missed when only the wooden sticks would have been investigated. However, this proportion decreased with an increasing number of eggs per capture event. We can therefore conclude that, although the container wall is also regularly used to oviposit eggs, the probability of overlooking the presence of container-breeding *Aedes* mosquitoes by examining the eggs laid on the wooden mouth spatula is very low.

Development of an artificial intelligence-based workflow for the reliable identification of mosquito species

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The rapid spread of invasive mosquitoes and the diseases they carry, accelerated by globalisation and climate warming, caused outbreaks of mosquito-borne illnesses in regions that were previously unaffected. In response, the importance of vector surveillance has grown significantly in recent years. Accurate identification of mosquito species is essential to evaluate their medical relevance. However, this task often requires specialised entomologists and costly laboratory equipment. Recent research has highlighted the effectiveness of Convolutional Neural Networks (CNNs) in precisely classifying mosquitoes based on their physical features and body images to overcome this challenge. Against this background, we have developed a classification algorithm tailored for vector surveillance and research programmes. Our goal is to simplify the identification of mosquito species, especially in areas with limited access to expert capacity and advanced laboratories. Additionally, we have explored ways to address model confidence, allowing CNNs to recognise uncertainties in predictions or account for mosquito species that are not included in the training dataset. To train our CNNs, we utilised a dataset consisting of 3800 mosquito body images primarily taken from literature sources and 8700 wing images generated through previous studies of our research lab. The resulting CNNs have demonstrated consistent and reliable classification capabilities for 15 mosquito species using wing images and 8 species using body images. The balanced accuracy for both types of images exceeded 95%. Furthermore, the algorithm possesses the ability to reject classifying new and previously unseen species. The integration of CNNs into entomological practice holds great promise in driving significant advancements in vector surveillance, enabling the monitoring of mosquito-borne threats and more precise and timely interventions. Through our work, we further aim to facilitate the application of artificial intelligence methods in infectious disease research, ultimately working towards improving public health outcomes.

Track the Tiger: unraveling resting site preferences of *Aedes albopictus* in Occitania, France.

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The Asian tiger mosquito, *Aedes albopictus*, thrives in urban environments by exploiting artificial containers for larval breeding. Despite the importance of adult mosquitoes' resting sites for public health preventative and curative measures, limited attention has been given to their exploration in temperate and European contexts. Correlations have been reported between vegetation and vector density at different developmental stages, but the role of urban vegetation elements, such as gardens and hedges, as resting sites for mosquito populations remains uncertain. Our study represents a comprehensive investigation into the resting sites of adult *Ae. albopictus* mosquitoes on a fine scale. It will help understanding whether recently emerged adults or older and potentially infectious females can be found in specific environments; a crucial information for practical vector control planning. Our objectives encompass: 1. identifying and characterising these resting sites; 2. phenotypic profiling of mosquito populations occupying these sites; and 3. establishing standardised indicators to enhance targeted preventive and reactive control strategies. We studied a total of 75 resting sites within a village of a 100 ha, encompassing both natural and artificial habitats. During more than 700 resting site sampling replicates we recorded local and global climatic conditions (temperature, wind, humidity, etc.) and vegetation characteristics. We used a combination of mosquito collection methods. Active females were captured using a 5-min human aspiration technique, followed by 1:30 min of aspiration per resting site, during periods of low mosquito activity. Dissections of adult mosquitoes allowed us to observe key anatomical markers indicating vector age structure, including ovarian follicle development (Christopher stages), parity, and spermatheca fillings in females, as well as genitalia rotation in males. Additionally, population dynamics were monitored using 30 ovitraps from April to December 2023. These results will be completed by ground releases of batches sterile male mosquitoes to further document resting site preferences and dispersal patterns. This research enhances our comprehension of *Ae. albopictus'* ecology and behaviour within urban landscapes. The identification of preferred resting sites will empower vector control operators, modelers, and decision-makers to adapt protocols and strategies, fostering more sustainable and efficacious management of public health associated with *Ae. albopictus* in the area.

Mosquito species identification with geometric morphometrics and CNNs based on wing images

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Accurate species identification is necessary to assess the medical relevance of a mosquito specimen, but it requires intensive experience of the observers and well-equipped laboratories. Herein, we would like to present two alternative methods to distinguish mosquito species based on images of their wings: geometric morphometrics and convolutional neuronal networks (CNN). Thereby, we focussed on native and exotic *Aedes* mosquitoes collected at different sites in Germany. In a first study, we analysed the potential of geometric morphometrics to distinguish *Aedes japonicus* and *Aedes koreicus*. The two species are closely related and difficult to distinguish by classical taxonomy. The wings of 271 specimens were removed, mounted and photographed. Subsequently, coordinates of 18 landmarks on the vein crosses were digitalised. The resulting two-dimensional landmark coordinates were used to analyse differences in the wing size and shape between *Ae. japonicus* and *Ae. koreicus*. While the wing size would not allow to discriminate the two species, the wing shape clustered species-specific and a leave-one-out validation resulted in a reclassification accuracy of 95% for the females and 91% for the males. In a second study, we analysed the potential of CNNs for species identification. A CNN is a deep learning algorithm to analyse visual data by mimicking the structure and function of the visual cortex. The data set consisted of 1,155 wing images from seven *Aedes* species, including the exotic species *Ae. albopictus* und six native *Aedes* species, as well as 554 wings from different non-*Aedes* mosquitoes. The wing images were used to train a CNN to differentiate between *Aedes* and non-*Aedes* mosquitoes and to classify the seven *Aedes* species. Image processing, data augmentation, training, validation and testing were conducted in python using the deep-learning framework PyTorch. The best-performing CNN configurations achieved an accuracy of 100% to distinguish *Aedes* from non-*Aedes* mosquito species and an accuracy of 96% to distinguish the seven *Aedes* species. In conclusion, geometric wing morphometrics and CNNs can provide a reliable method to distinguish closely related *Aedes* species by means of wing images. Future possibilities for practical application as well as the advantages and disadvantages of both methods will be discussed.

Invasive mosquito species and their (mis)management in Germany

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During the last 15 years, five invasive mosquito species succeeded in establishing in Germany: *Aedes albopictus*, *Ae. japonicus*, *Ae. koreicus*, *Culiseta longiareolata* and *Anopheles petragnani*, with the first three of them being potential vectors of disease agents. Additional invasive species were reported but did not establish: *Ae. aegypti*, *Ae. berlandi* and *Ae. pulcritarsis*. All species were detected by the national mosquito monitoring programme, consisting of trapping, the citizen science project 'Mueckenatlas' and manual larval/adult sampling, and some of them also by collection activities carried out regionally on federal state level. Findings of invasive mosquito species made within the framework of the national monitoring programme are generally made public online or by scientific articles, but findings of *Ae. albopictus* and *Ae. aegypti* are promptly being reported via the German National Expert Commission 'Mosquitoes as Vectors of Disease Agents' to the affected federal and communal authorities or, if available, to state units/contact persons appointed by federal state authorities to deal with invasive mosquitoes. Subsequent measures are in the responsibility of federal states but are not mandatory as long as there is no evidence of pathogen transmission by the invasive mosquito species. Due to a lack of expertise, personnel and money, local reactions are hesitant and often insufficient, although the mosquito expert commission can be consulted for theoretical and practical advice. However, awareness and preparedness are slowly increasing on state level, and surveillance and control are finally implemented in most cases when local *Ae. albopictus* reproduction has been demonstrated. The presentation will provide an update on invasive mosquito species detections in Germany made within the authors' sphere of activity and sketch their reporting and management by responsible authorities.

Evaluating spatial and temporal covariate resolution in surveillance and modelling of mosquitoes

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Mosquito-borne diseases pose significant one health risks, making surveillance and modelling of mosquito populations a critical endeavour for public and animal health. Effective surveillance strategies are essential to understand and combat the spread of invasive mosquito species while reducing the potential threats associated with vector-borne diseases. The integration of covariates, such as environmental factors and climatic variables, into mosquito surveillance and modelling efforts is fundamental for gaining insights into the dynamics of mosquito populations. However, the determination of the appropriate spatial and temporal resolutions of these covariates remains an ongoing challenge and subject of debate within the scientific community. In this presentation, we use the island of Guadeloupe, a French overseas region located in the southern Caribbean Sea, as a case study. Guadeloupe has been chosen as the study area due to its vulnerability to mosquito-borne diseases and therefore the importance of mosquito surveillance in this region. Avia-GIS has been conducted longitudinal mosquito field monitoring on the island. The focus of our study is to explore the correlation between mosquito field data collected in the vicinity of residential areas and various covariate datasets obtained from different sources, each characterised by distinct spatial and temporal resolutions. These covariate sources encompass a wide range, including data from local weather stations to satellite-based products. Our research specifically examines mosquito activity patterns and the mosquito comfort zones with respect to the different covariate sets. By analysing the interplay between environmental factors, climatic variables, and mosquito populations, we aim to shed light on the complex relationships governing mosquito dynamics in Guadeloupe. This work is an integral part of the ongoing Avia-GIS MosqACT project, funded by VLAIO (Flanders Innovation & Entrepreneurship). The overarching objective of this project is to provide a modular IoT-powered set of tools based on the improved vector population dynamics model and disease models developed by Avia-GIS. To provide better protection from mosquito borne diseases through tailored vector control and to allow in-silico testing of what-if scenarios in an easy-to-use framework. By achieving these goals, we seek to provide valuable insights that can inform a wide range of mosquito surveillance and modelling efforts, ultimately enhancing our capacity to mitigate the risks posed by mosquito-borne diseases. The findings of this study holdt a deeper understanding of the relationship between covariate resolution and mosquito dynamics can lead to more effective monitoring and modelling efforts.

Remote monitoring of disease vector mosquitoes using smart traps with a novel optical sensor

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Mosquitoes can transmit viruses such as dengue, West Nile, Zika or Chikungunya. The prevention of vector-borne diseases largely depends on effective and sustainable vector surveillance, although the activities foreseen in entomological surveillance programmes demand high costs with specialised human resources and with the logistics that is invested in the periodic inspections of insect traps. The implementation of sustainable vector surveillance strategies is essential. Field sensors coupled to standard suction traps were deployed in three locations in Portugal for real pilot trials during the mosquito seasons. The catch bags of the traps were checked weekly and then compared with the automatic classification obtained from the field sensor. The relationship between manual and sensor counts was assessed using Pearson correlation analysis and linear regression analysis. Positive correlations (r>0.9, p-value <0.05) between the number of four mosquito classes (*Aedes* female, *Aedes* male, *Culex* female and *Culex* male) counted by the sensor and by manual inspection was observed in all trials. Linear regression analysis showed a good fit of the linear regression line to the data points of manual counts versus sensor counts per collection cycle: R^2=0.813 for all *Culex* and *Aedes aegypti* in the Madeira field trial, and R^2=0.9809 and R^2=0.9752 for all mosquitoes counted manually and by the sensor on the other two field trials. The strong positive correlation and the good-fit linear regression data between the manual and automated counts makes the use of this sensor a valuable automated remote mosquito monitoring tool opening new future perspectives in mosquito vector surveillance. This work has been developed within the VECTRACK (H2020-EIC-FTI-2018-2020) and MOBVEC (Ref. 101099283 – MOBVEC – HORIZON-EIC-2022-PATHFINDEROPEN-01) projects funded from by the European Commission.

Lessons learnt from invasive mosquito species control in Belgium

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The first invasive mosquito species (IMS) that was detected in Belgium was *Aedes albopictus* in 2000 and was linked to introductions in France. Since 2007, active monitoring of IMS in Belgium is implemented through short-term projects. From 2013 until today, *Ae. albopictus* has been consistently detected nearly every year. First it was only detected at used tyre and lucky bamboo import companies. In 2018 the first introductions were noted at parking lots along highways. Finally in 2022 the species was found in private gardens. Additionally, in Belgium, in 2002 a locally established population of *Aedes japonicus* was found and in 2008 *Aedes koreicus* was detected for the first time. The latter was the first report of the species in Europe. The control of IMS is a regional competence in Belgium, hence its implementation differs depending on the region (Flanders, Brussels, Wallonia). In Flanders, control of *Ae. albopictus* was implemented ad hoc by non-professionals between 2014–2016, but since 2016, a private company is hired to perform the control operations. In Wallonia, the control of *Ae. albopictus* started in 2020 despite earlier detections. In general, control of *Ae. albopictus* was applied in response to a detection and since 2016 in some cases also as a preventive measure e.g. at the beginning of the season. As the locations of introduction expanded, now including private gardens, the implementation modalities changed with an intensive door-to-door control campaign after the detection of *Ae. albopictus* in private gardens in 2022. The control of *Ae. japonicus* was implemented from 2012 onwards initially to eliminate the species in Belgium. Also, control of *Ae. koreicus* was implemented between 2019 and 2022. The mosquito control capacity in Belgium has grown over the last decade. Yet, the increasing number of *Ae. albopictus* introductions in urban areas bring new challenges on top of existing ones. In general, the mosquito surveillance and control in Belgium would benefit from an overall plan within a legal framework in which objectives, targets, methods, responsibilities and budget of both surveillance and control are defined. That way, the country would be better prepared for the increasing expansion of IMS and their potential public health threat.

Comparison between different methods to measure the density of *Aedes albopictus*

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To define the density of a population of mosquitoes is very important to establish the opportunity of an adulticide application and to measure its efficacy, to evaluate the risk for an outbreak of a Mosquito Borne Disease (MBD), to understand the quality of a running project. While for some species (*Anopheles* spp, *Culex* spp, *Ochlerotathus* spp, *Aedes vexans*, etc.) the dry ice trap is very effective and represents a standard method, for *Aedes albopictus* (Aa) there are many different methods but no one can be considered the best, for the several issues each of them has. In this work the Human Landing Counting (HLco), the Estimated Flying Mosquitoes (EFM), the BG-Sentinel trap (BG-s), the BG-Gat trap (BG-g) and the Ovitraps (OT) have been compared to investigate merits and defects. To be able to define the population of Aa independently of the used method, a conversion factor between the methods has been investigated. The study was conducted in three naturally infested areas. First, the volunteer reached the infested site, performing the HLco for 5 minutes and estimating at the same time the number of mosquitoes flying around his legs. Once the assessment was concluded, the Bg-s baited with the attractant Bg-lure, was placed for 48 hours. In the same site an Ovitrap or the Bg-g were alternatively placed for 7 days. The test was conducted for the along summer season, with the same volunteer, performing the HLco always at the same hour of the day. Based on the obtained data, the five methods show the same trend to measure the Aa density for the whole summer season. A conversion factor can be calculated. The collected data permitted to underline qualities and issues of each method, defining the best method depending of the specific conditions and purposes.

Addressing geographic gaps in UK mosquito surveillance: investigating mosquito distributions in Scotland

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As key mosquito vector species and mosquito-borne pathogens are detected at increasingly higher latitudes, it is vital that mosquito surveillance programmes are implemented in northern Europe to monitor mosquito species distributions and enable early detection of circulating pathogens. In the United Kingdom, surveillance activities have largely focused on southern areas of the country, leaving a knowledge gap regarding vector-borne disease (VBD) risk in Scotland. To address this gap, the 'Mosquito Scotland' project aims to characterise mosquito species distributions, abundance, ecology and pathogen prevalence across Scotland. Here, we present findings from the first season of entomological surveillance in Scotland. Sampling was conducted between June and October 2023 at 21 geographically dispersed sites comprising six wetland types: coastal saltmarsh, wet grassland, wet woodland, reedbeds, ponds and blanket bog. BG-Pro traps baited with carbon dioxide and 1-octen-3-ol were run at 48-hour intervals at each site on a monthly basis. Larval dipping was carried out in aquatic habitats at each site. Environmental conditions (ambient temperature and relative humidity) and hydrological variables (water temperature, depth, pH, salinity and conductivity) were measured at each sampling event. Over 1500 adult mosquitoes were collected between June and the end of August. At least sixteen species were morphologically identified, including high proportions of flavivirus vector *Culex pipiens* s.l./*Cx. torrentium* and potential bridge vector *Coquillettidia richiardii*. The predominant taxa captured at the adult stage were *Cq. richiardii*, *Cx. pipiens* s.l./*Cx. torrentium*, *Anopheles claviger* and *Aedes detritus*. *Cx. pipiens* s.l./*Cx. torrentium* and *Culiseta annulata* were the most abundant species at the larval stage. *Cx. pipiens* s.l./*Cx. torrentium*, *Cq. richiardii*, *An. claviger* and *Cs. annulata* were widely distributed in a broad range of habitats, while *Ae. detritus* was locally abundant in areas of coastal saltmarsh. Mosquitoes were found at 20 of 21 sites, including in high numbers at the most northern latitudes. Preliminary observations suggest that the abundance of native vector species is not latitudinally constrained, challenging widely-held assumptions about the scarcity of mosquito vectors in Northwestern Europe. The data collected here will be used to develop species distribution models and disease risk maps that will provide greater insight into risk factors for the establishment of VBD in Scotland.

Estimating Asian tiger mosquito dispersal through genetic analyses

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Understanding vector dispersal is critical for the control and management of vector-borne diseases. Traditionally, it has been studied through mark-release-recapture methods, which are logistically demanding and may not represent the actual behaviour of the vectors under their local conditions. Genetic analyses represent an excellent opportunity to monitor mosquito dispersal in the same conditions that they experience, yet previous studies have inadvertently overlooked the bidimensional nature of mosquito movement. In response, we have developed a comprehensive framework, building upon the work of Filipovic *et al.* (2020), to address these challenges. Here, we used genetic-based kinship estimates to assess Asian tiger mosquito (*Aedes albopictus*) dispersal in a highly urbanised landscape within the metropolitan area of Barcelona (Catalonia, Spain). We observed 12479 kinship relationships between 428 mosquitoes, which allowed us to estimate a Weibull bidimensional dispersal kernel with scale parameter 110.3 and shape parameter 1.323. The resultant mean dispersal distance of 160.6 meters falls within the typical dispersal range of *Ae. albopictus*, albeit at the lower end of the spectrum. This research not only sheds light on the bidimensional aspects of mosquito movement but also paves the way for future investigations into various forms of mosquito movement (e.g. skip oviposition) and the underlying determinants of mosquito dispersal at micro-spatial scales. Additionally, our findings underscore the utility of genetic analyses as a valuable alternative to traditional mark-release-recapture methodologies. This work enhances our ability to characterise and comprehend mosquito movement patterns, with implications for the effective management of vector-borne diseases.

Blood-feeding preferences of *Aedes albopictus* in laboratory conditions

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Aedes albopictus is a well-known mosquito species due to its worldwide implication as a vector of several diseases of significant public health relevance. This species is established in a large number of cities in Spain, as well as in many other European territories. It prefers to feed on human than other hosts, however most studies have been performed under conditions with limitations on hosts availability or under different laboratory variables. In this study, the blood-feeding preferences of *Ae. albopictus* were analysed using Hemotek under laboratory conditions. Several assays were conducted using human, rat and cow blood, comparing treatments with and without EDTA by using different feeding membranes. Five variables were measured: percentage of females feeding, fecundity, oviposition, adults survival and the viability of eggs. Despite the results did not demonstrate a clear preference for human blood, it was observed that human blood followed by rat blood are the most recommended for optimising the rearing of *Ae. albopictus* in the laboratory; since they provided the highest values of feeding, fecundity, and egg viability. Parafilm was the membrane that showed the highest percentage of *Ae. albopictus* feeding. The use of EDTA or other anticoagulants in blood is not recommended for mosquito feeding since this product influences on several variables, such as the number of eggs obtained. Our study contributes to the knowledge and learning of mosquito rearing techniques in the laboratory to improve efficiency and productivity.

Swiss national monitoring programme of invasive *Aedes* mosquitoes: strategy adaption after 10 years

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Over the past 10 years, we set about 150 oviposition and 20 BG-Sentinel traps at over 40 sites in order to monitor the introduction of *Aedes albopictus* and other invasive *Aedes* species along the motorways and a few additional points of introduction into Switzerland. During that time we repeatedly detected *Ae. albopictus*, *Ae. japonicus* and *Ae. koreicus* both south and north of the Alps. While the detection patterns suggest *Ae. albopictus* being primarily passively dispersed along the motorways, originating from the South, *Ae. japonicus* seems to have spread more actively and radially from its original point of introduction. Being national, the monitoring programme has sent out an important 'political' message and has encouraged several cantons to initiate their own, regional surveillance programme. Moreover, it served as the foundation for building the Swiss Mosquito Network, a network that coordinates all surveillance efforts across Switzerland. However, after 10 years of monitoring along the motorways, the national monitoring programme's added scientific value has decreased as the dispersal along the motorways is now well established. Yet, to keep its national 'knitting' character, we re-designed the trap network and relocated the traps from the motorways to other points of introduction with a focus on campgrounds and long-distance bus terminals using a set of well-defined selection criteria. In contrast to the motorway system, the new trap network is more balanced across the whole country, which may further encourage cantons to initiate their own programme. At the meeting, we will present the first data from the 2023 mosquito season and discuss the challenges and opportunities of the rebuilt Swiss national monitoring programme.

Development and validation of a MidInfraRed Spectroscopy approach for *Aedes albopictus* age grading

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Quantifying mosquito population age-structures is crucial to assess the dynamics of mosquito-borne-diseases and the effectiveness of vector control interventions. This is especially the case when transmission of arboviruses is not endemic and the assessment cannot be based on changes in human cases. However, determining the age of a mosquito vector is strongly hampered by the need to rely on labour-intensive ovarian dissections by well trained personnel. Many efforts have been placed in recent years to overcome this major constraint, particularly for major tropical vector species. One promising approach is based on mid-infrared spectroscopy (MIRS) and machine learning (ML), which can quickly and accurately determine the age of female *Anopheles* African malaria vector species based on the measurement of biochemical changes in the cuticle over time. Here, we show the results obtained by applying MIRS-ML for age determination of adult *Aedes albopictus* females and males from laboratory and semi-field settings. Field collected *Aedes albopictus* eggs were reared to adulthood either under laboratory or semi-field conditions. Adult females were regularly blood-fed and allowed to lay eggs. Males and females at different physiological states were collected every 3 days until day-36 under laboratory conditions, and every 3 consecutive days (from 1–3 to 31–33 day old) in case of semi-field adults. Spectra from individual mosquitoes was acquired by Attenuated Total Reflection FT-IR spectroscopy using a Bruker ALPHA II spectrometer between 4000 and 400 cm^{-1} with 4 cm^{-1} resolutions. The dataset was split into training and test sets (80–20%). Several machine learning algorithms were tested, and logistic regression (LR) was selected for optimisation based on the accuracy of the predictions. Under laboratory conditions, LR model predicted the mosquito age of both male and female mosquitoes with 95% accuracy, ranging from 86% to 100%, depending on the age-group. We are currently analysing spectra of semi-field reared mosquitoes, where preliminary results suggest that age-grading is possible also under these more variable conditions. These results support the high potential of MIRS-ML as the first non-morphological approach to accurately and cost-effectively assess the age structure of *Ae. albopictus*. Further studies are needed to confirm its reliability on predicting field-collected specimens.

Session 1. Surveillance and management of invasive mosquito species

POSTERS

Contrasting *Aedes albopictus* and *Aedes japonicus* egg laying activity in 2021–2022 in northern Spain

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Climate change impacts *Aedes* Invasive Mosquitoes (AIM) establishment, favouring reproduction in warmer temperatures. However, different AIM species have varying preferred temperature ranges, responding differently to climatic variations. Analysing patterns across different years can reveal underlying drivers of population fluctuations. This study presents preliminary data on contrasting egg laying between 2021 and 2022 in northern Spain, where *Aedes albopictus* (*Aea*) and *Aedes japonicus* (*Aej*) have established populations. The difference in the mean egg counts (MEC) of two AIM species in the Basque Country was evaluated using ovitraps. In the context of the AIM Surveillance Programme of the Basque Country, data from 9 and 13 municipalities with *Aea* and *Aej* established populations, respectively, was analysed. Oviposition sticks were replaced every 14 days and examined for egg counting. Positive sticks were immersed in dechlorinated water and emerged larvae were placed in breeders until becoming adults for identification. In 2021, the MEC for *Aea* and *Aej* were 7.89, and 4.24, respectively. However, during 2022, the MEC for *Aea* showed a significant increase, reaching 27.24 eggs (p=0.004). In contrast, the MEC for *Aej* slightly declines without significant differences (MEC=3.13; p=0.3). The highest MEC values for *Aea* were observed at the end of August in 2021. In contrast, in 2022, the highest values were observed in September, surpassing the highest values of 2021 even in October and early November. *Aedes japonicus* egg laying was more or less constant until October in 2021. However, in 2022, an apparent decrease was observed from the end of July. The 2022 has an unusually hot summer with mean summer temperature 1.8°C higher than 2021. Elevated temperatures may favour *Aea* reproduction and population growth, while *Aej* seems to have a reduced adaptability to such extreme temperature changes. Further studies, with data from more years and association with different climatic variables, would be interesting to better understand these contrasting dynamics. In conclusion, the differential responses of AIM species to climatic fluctuations highlight the necessity for adaptive and context-specific mosquito control measures in response to changing climatic conditions.

Comparison of a multiplex PCR with DNA Barcoding for identification of container breeding mosquito species

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Identification of mosquitoes greatly relies on morphological examination under a microscope. Since some species cannot be distinguished reliably by morphological methods, or at least not at certain stages, it is important to incorporate molecular techniques into the diagnostic pipeline. DNA barcoding using Sanger sequencing is currently widely used for identification of mosquito species. However, this method does not allow detection of multiple species in one sample, which would be important when analysing mosquito eggs. Collection of container breeding *Aedes* eggs is typically performed by using ovitraps, which consist of a black container filled with water and a wooden spatula plunged inside for oviposition support. *Aedes* mosquitoes of different species might lay single or multiple eggs on the spatula. In contrast to Sanger sequencing, multiplex PCR protocols targeting the specific species of interest can be of advantage for detection of multiple species in the same sample. For this purpose, we adapted a previously published PCR protocol for simultaneous detection of four different *Aedes* species that are relevant for Austrian monitoring programmes: The invasive species *Aedes albopictus*, *Aedes koreicus* and *Aedes japonicus*, as well as the native species *Aedes geniculatus*. For evaluation of the adapted multiplex PCR protocol, we analysed 2290 ovitrap mosquito samples from the years 2021 and 2022, which were collected within the scope of an Austrian nationwide monitoring programme. We compared the results of the multiplex PCR to the results of DNA barcoding and to the morphological examination. Of 2290 samples, 2011 could be identified using the adapted multiplex PCR while species determination using DNA barcoding of the mitochondrial cytochrome c oxidase subunit I gene was possible in 1774 samples. The adapted multiplex PCR showed a mixture of different species in 73 samples, which could not be detected with DNA barcoding. In conclusion, identification of container breeding mosquito species in ovitrap samples was more successful when using the multiplex PCR protocol as opposed to the DNA barcoding protocol. Additionally, the multiplex PCR allowed us to detect multiple species in the same sample, while those species might have been missed when using Sanger sequencing alone. Therefore, we propose that the multiplex PCR protocol is highly suitable and of great advantage especially when analysing mosquito eggs from ovitraps.

Entomological surveillance of *Aedes albopictus* and arbovirus imported cases in Eastern Spain throughout 2022

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The Laboratory of Entomology and Pest Control of the University of Valencia, hired by the regional government of the Generalitat Valenciana, have carried out the entomological surveillance of the so-called Asian tiger mosquito (*Aedes albopictus* (Skuse, 1894)) along the year 2022 in Valencian Autonomous Region located in Eastern Spain, with the exception of the city of Valencia which has been executed by a private pest control company. The main purpose of this plan of action was focused not only on continuing the detection and monitoring of this nematoceran mosquito species populations in the Valencian municipalities free of this culicid mosquito species until the end of 2021, but also in the fulfilment of entomological surveys after imported arboviruses cases notified by the regional health system. As a result of the implemented methodology, consisting of having realised an active searching and detection of their particular breeding sites in both urban and peri-urban environments through the provinces of Alicante, Castellón and Valencia, this laboratory has concluded the absence of new populations of this allochthonous mosquito species in all those municipalities free of this dipteran in December of the previous year. Likewise, this laboratory has been in charge of conducting the mosquito entomological surveys in 31 municipalities of the three provinces that make up this autonomous community, after the declaration of 33 cases of arthropod-borne diseases due to the Chikungunya, dengue and Zika viruses, which were reported in travellers who returned from several countries of Latin America, Asia, and West Africa. As a consequence of the risk of being transmitted by *Ae. albopictus*, the methods used to eliminate their populations as well as to minimise the probable transmission of these viruses at local level to other human beings were focused on detecting the presence of tiger mosquito populations, not only of imagoes but also of larvae, in the surroundings of the affected citizens residence to authorise the implementation of suitable treatments of the registered breeding sites. The conclusion of this second part of the fulfilled plan lies in the finds of tiger mosquito populations, of both preimaginal and imaginal stages, in 18 municipalities out of the 33 imported arbovirus cases that were informed in 2022.

The use of thoracic chaetotaxy as a tool for identification of *Aedes* invasive species

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Globalisation facilitates the spread of *Aedes* invasive mosquitoes (AIM) and their associated mosquito-borne pathogens in Europe. The monitoring of AIM is conducted in many countries for early detection and control. During the life cycle, adult mosquito specimens may lose their scales and setae that are important characters for morphological species identification. Moreover, automatic adult traps can result in mosquito catches in a relatively poor state of preservation. Mosquitoes often lose their body parts and it becomes difficult or impossible to identify mosquitoes by morphology. Molecular species identification is a quite expensive technique, not available or limited in many countries. Therefore, here we intensively test thoracic chaetotaxy as a technique for correct and rapid identification of adult mosquitoes, based on the structural characters, which would not be lost and less damaged during the sampling. Thorax is usually intact in mosquitoes collected by different methods. Setal follicles are present on thorax even if setae are missing making possible the study of their topology and quantity. Thus, different populations of *Aedes albopictus*, *Aedes aegypti*, *Aedes japonicus* and *Aedes koreicus* females and males were used to provide information on the number and localisation of setae in different thoracic setal groups (upper and lower mesepimeral, upper and lower mesepisternal, prealar, postpronotal, postspiracular, propleural and supraalar). Comparative analysis of setal patterns among invasive mosquito species and evaluation of their taxonomic importance were performed. In addition, AIM thoracic chaetotaxy was compared with other European and Afrotropical *Aedes* species (*Aedes geniculatus*, *Aedes simpsoni* s.l., *Aedes cumminsii*, *Aedes furcifer*, *Aedes jamoti*, *Aedes punctor*, *Aedes rusticus*, *Aedes sticticus*, *Aedes vexans* and *Aedes vittatus*), which may occur in adult catches together. The method appeared to be highly suitable to identify mosquitoes, while the clearest differences were found between mosquitoes belonging to different subgenera.

Invasive mosquito surveillance in the UK, 2020–2023

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Multiple countries across Europe have established populations of *Aedes albopictus* and have been responsible for serve nuisance biting and multiple vector-borne disease outbreaks. Due to the risk of these mosquito entering the UK, the United Kingdom Health Security agency (UKDOMHSA) has been carrying out invasive mosquito surveillance since 2010. Here we update on the last 3 years of the surveillance between 2020–2023in the UK. The aim is to coordinate national surveillance activities and detect any incursions of *Ae. albopictus* and to determine whether any populations have become established. The surveillance relies on local stakeholders including local environmental health teams, port health officers, Royal Air Force and Animal and Plant Health Agency staff. The surveillance started in 2010 with 11 sites taking part and increased to 24 sites in 2020 and 2021, 42 sites in 2022 and 58 sites in 2023. Sites are chosen based on a risk-based approach with the focus on ports of entry and along main highway routes. These include 5 airports, 25 seaports, Eurostar St Pancras and 21 service station and truck stops. At each site at least 5 ovitraps with water and polystyrene blocks were collected every two weeks from June to October. Water samples were sent to UKHSA for checking for eggs and larvae. In addition to the 58 sites, 20 sites were run by MEZE in London and along the south coast of the UK between July and October. Using the same method, traps were placed in urban and suburban areas, to assess whether previously unknown populations of *Ae. albopictus* had become established. Between 2020 and 2023 no populations or incursions of invasive mosquitoes were detected at any site. This project supplements UKHSA's other mosquito surveillance and monitoring activities, including a passive citizen science mosquito recording scheme and a questionnaire asking local authorities about reports of mosquitoes.

Genetic diversity and wing geometric morphometrics among four populations of *Aedes aegypti* from Benin

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The global impact of the arbovirus vector *Aedes aegypti* is a major concern for medical and public health, affecting millions of people worldwide. Originally native to Africa, this species has spread throughout the world. While the genetic makeup of *Ae. aegypti* has been extensively studied in the New World, limited knowledge exists regarding its genetic diversity in Africa, particularly at a microgeographical level. In this study, we investigated the mitochondrial cytochrome oxidase I of four *Ae. aegypti* populations from Benin and employed wing morphometric analyses as a cost-effective and reliable tool to explore population structure. Our sampling encompassed various sites in Benin, spanning from the southern to the northern border, including urban, semi-urban, and sylvatic areas. We observed a notable level of genetic diversity (Hd=0.8333) and nucleotide diversity (π= 0.00421986), identifying seven distinct haplotypes. Sylvatic and semi-urban sites exhibited a greater number of haplotypes compared to urban sites. Utilising 18 wing landmarks, we calculated the centroid size, revealing a significant variation among the three landscape types. However, principal component analysis, employed to assess wing shape variation, did not demonstrate significant differentiation between populations based on landscape type. Our findings indicate a substantial genetic and morphological diversity among *Ae. aegypti* populations in Benin, providing insights into important biological characteristics and the potential of different populations to transmit viruses. To the best of our knowledge, this is the first study in Africa that integrates genetics and morphology to analyse the population structure of the crucial arbovirus vector *Ae. aegypti.*

First observations of *Aedes albopictus* (Skuse 1894) in Sweden

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During the mosquito season of 2023 the National Veterinary Institute in Sweden have been running ovitraps in three municipalities in Skåne (Malmö, Helsingborg and Trelleborg). The project was set up as a pilot project for building a surveillance system for invasive mosquitoes in Sweden. We used 45 0.4 l black plastic cups filled with hay infusion and a wooden spatula. In each municipality we set up five traps in three different sites. The emptying and maintenance of the traps was assigned to staff from the municipalities who sent the spatulas to SVA biweekly from the beginning of June to the beginning of September. We chose truck stops and public campsites as trap sites. Identification was made with species specific qPCR. We also announced the project in national media to raise awareness of invasive mosquitoes. On August 24th an email was received from a family in Stockholm who had found larvae in a glass bowl containing plants they had brought back from a vacation in Spain. The 15 larvae and 1 pupa were collected the day after and brought to SVA for identification. The day after collection a male *Ae. albopictus* emerged from the pupa. This constitutes the first documented observation of *Ae. albopictus* in Sweden. In the municipality of Trelleborg species specific *Ae. albopictus* DNA was registered from material collected from a trap set between June 15th and June 27th. The trap site was in an industrial area close to the harbour where trucks from all of Europe park overnight. This constitutes the second observation of *Ae. albopictus* in Sweden. With the abundant traffic of trucks, mobile homes and private cars between Europe and southern Sweden we anticipated to find invasive mosquitoes in southern Sweden at some point. The results were surprising in that even with quite low effort we found two separate introductions of *Ae. albopictus* to Sweden. We believe that a combination of traps and heightened awareness and citizen participation is a good strategy to intercept introductions of invasive mosquitoes. We hope that we will be able to continue and expand this project to other municipalities in southern Sweden with traffic from European countries.

The rise of *Aedes albopictus* in the Basel region at the Swiss–French–German tri-national border

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Basel is located in the Upper Rhine Valley bordering France and Germany. In the city of Basel, *Aedes albopictus* was detected for the first time through a report from a citizen in 2015. Subsequently, the canton of Basel-Stadt, together with Swiss TPH, has implemented a strategy consisting of preventative measures, monitoring and control which has been intensified since 2020. The monitoring combines a network of oviposition traps and a citizen science approach by which citizens are encouraged to report suspected *Ae. albopictus* specimens through the Swiss Mosquito Network. The prevention and control includes larval source management by treating catch basins with larvicides and public participation in clearing and avoiding breeding sites on private grounds. A similar approach is taken by the neighbouring Canton of Basel-Landschaft. Since 2017, the population density of *Ae. albopictus* and the size of the infested area have been growing with an unprecedented increase in recent years. We will present the history and the status of *A. albopictus* expansion in the Basel region, methods deployed and will discuss current and future challenges and potential strategies.

Uncovering *Aedes albopictus* spatio-temporal dynamics at urban microscales through genetic and network analyses

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The Asian tiger mosquito (*Aedes albopictus*) is an invasive vector of disease pathogens that constitutes a major public health concern. The species shows a preference for highly populated and urbanised areas which, coupled with its marked anthropophagic behaviour, makes it crucial to implement effective surveillance and control strategies. In this context, inferring spatio-temporal patterns of mosquito distribution, which can be derived from genetic-based relatedness estimates among sampled individuals, is critical for ensuring the success of mitigation activities. Here, we screened 428 adult female *Ae. albopictus* for 18 microsatellites and combined genetic and network analyses to uncover spatio-temporal patterns of mosquito distribution in a highly urbanised landscape within the metropolitan area of Barcelona (Spain). Our analysis revealed that 43% (184) of the collected mosquitoes exhibited close-kin relationships. These mosquitoes formed a complex relatedness network comprising 47 distinct modules, with 13 modules grouped in a single component. While modules showed high temporal dissimilarity, no spatial pattern emerged when considering the location of close-kin mosquitoes. The trap monolayer network (incorporating spatial information only) did not detect any spatial pattern between sampled traps, based on the number of shared close-kin mosquitoes between pairs of traps. Similarly, the trap multilayer network (integrating both spatial and temporal information) indicated a high turnover of modules assigned to each trap. Taken together, results suggest that mosquitoes in urban environments exhibit effective local-scale dispersion across multiple generations, and point to a lack of urban or landscape elements significantly influencing mosquito dispersal within our study area.

Estimating the number of female mosquitoes from the number of eggs

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Over the last few decades, mosquito-borne diseases (MBDs) are increasingly emerging and re-emerging worldwide as a consequence of the spread of many mosquito vector species due to globalisation of human trade and mobility. MBD risk assessment plays a crucial role in public health decision-making for the timely adoption of intervention measures. MBD risk is commonly evaluated using the basic reproduction number, R_0, possibly one of the fundamental and most often used epidemiological metrics for the description of the contagiousness or transmissibility of infectious and parasitic agents. One of the main factors determining the epidemic risk is the density of host-seeking female mosquitoes, a factor included in the calculation of R_0 for MBD. Female densities can be estimated measuring directly the number of biting females by the Human Landing Collection (HLC) method. As this method has several disadvantages, it has been proposed to use egg densities measured via oviposition traps (ovitraps) to predict female densities. Here, we tested whether eggs numbers of *Ae. albopictus* can be used to reliably predict female numbers. To do so, we used a large and comprehensive dataset of human landing data collected in several localities in southern Switzerland associated with the dense network of ovitraps used for standard monitoring in the same region. We aimed at creating a model that: 1. can be used to predict female densities from egg densities in the study area (i.e. 'local' model); and 2. can potentially be used outside the study region (i.e. 'global' model). In general, this type of model takes eggs numbers as input and returns the females numbers as output. The model may also take other variables as input (e.g. weather conditions) to achieve higher accuracies.

Spreading of invasive mosquito *Aedes japonicus* and *Aedes koreicus* in Italy

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In this study we report data on the occurrence and spread of *Aedes japonicus japonicus* and *Aedes koreicus* in Italy from 2011 to 2022. Mosquitoes were collected in the frame of different projects by larval search, traps for adult mosquito and ovitraps from March to November. Species identification was performed morphologically and molecularly by PCR and sequencing. Sites and municipalities were considered positive if larvae, adults or eggs (larval identification after hatching) were found. *Aedes albopictus* was not considered because this species is present all over Italy. During the last 12 years of entomological surveillance, 1703 municipalities of 7 Italian Regions (the whole of Northern Italy) were monitored. *Aedes koreicus* occurs in 456 municipalities (26.8%) and *Ae. j. japonicus* in 210 (12.3%). After its first finding in 2011 in Veneto Region, *Ae. koreicus* spread throughout northeast in five years; it was also found in Lombardy at Italian Swiss border. It reached northwest nine years later. A probably new introduction was recorded in Liguria region (northwest Italy) in 2015. *Aedes j. japonicus* was found in 2015 in one municipality bordering Austria and has spread through the North reaching the Northwest in 2019. To date, *Ae. j. japonicus* spread seems slower compared to *Ae. koreicus*. The expansion of both species southwards seems to be limited by the high mean summer temperatures and by the high density of the competitor species *Ae. albopictus* in the plain area. The overlapping of *Ae. koreicus*, *Ae. j. japonicus* and *Ae. albopictus* distribution is complicating the entomological monitoring system, due to their similar biology and morphology. Therefore, long-term surveillance and early detection are needed to limit the further spread and plan control actions against these invasive mosquitoes. This work was funded by the autonomous province of Trento and Veneto and Friuli Venezia Giulia Regions.

Tree-hole breeding mosquitoes in the Prater area in Vienna, Austria: do we find tiger mosquitoes there?

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Asian tiger mosquitoes (*Aedes albopictus*) have recently been documented in Austria in several provinces. In 2020 they were first reported in Vienna in an allotment garden in the Prater (a large public park). An ovitrap-based monitoring from 2022 indicates establishment and spread of the tiger mosquito but *Aedes japonicus* and *Aedes koreicus* have also been found in the entire Prater. The later mainly consists of renaturation areas where a lot of dead trees and tree holes are present. The highest egg abundances were documented in the locations between the main highway in Vienna and a railway bridge, as well as in a forested dog area, where almost no artificial breeding sites are present. In their native distribution area in Asia, tiger mosquitoes are well known as tree-hole breeders. However, in areas where tiger mosquitoes have been introduced, researchers and control activities focus mainly on small artificial containers, whereas those natural breeding sites are rarely considered. To evaluate the importance of tree holes as breeding sites in a non-native urban environment, more than 20 tree holes of various tree species (e.g. maples and linden) were examined for the presence of mosquito larvae during the season 2023. Larvae were sampled up to twice monthly with a nasal aspirator. Additionally, ten ovitraps were set up in this study area and checked weekly. Mosquito larvae and eggs were specified morphologically and further analysed with molecular techniques and barcoding to species level. Results of this currently ongoing study are presented in terms of diversity and abundance and indicate that tree holes might not be the preferred breeding habitats for tiger mosquitoes in this area.

Spread of the Asian tiger mosquito in western Switzerland: measures taken, successes and problematic cases

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The Asian tiger mosquito (*Aedes albopictus*) was first detected in Switzerland in 2003 in Ticino and in 2013 north of the Alps. Due to its rapid spread, the cantons of French-speaking Switzerland (Romandie) have started to implement surveillance programmes in 2019, with evidence of its presence already in three cantons. Since then, the number of findings through monitoring and citizen reports has steadily increased, which has led to established populations in almost three Cantons. In most cases, the control measures and information campaigns taken by the cantons have made it possible to slow down the spread of the tiger mosquito. Rapid site inspections in case of tiger mosquito findings in new areas, combined with immediate control measures, have made it possible in several cases to eradicate them before they could establish themselves. On the other hand, there are also negative examples where the cantonal authorities were not willing to take the necessary measures, which led to a rapid spread and increase in population densities of the tiger mosquito. Here, we will present the evolution of surveillance programmes in the cantons of western Switzerland over the past five years, with examples where the introduction of the tiger mosquito was successfully countered and others where it has been able to spread rapidly due to lack of intervention.

A journey through time: a decade of entomological surveillance in the Canary Islands

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The location of the Canary Islands (CI, Spain), halfway between Europe and Africa, makes it a risk area for the introduction and establishment of invasive *Aedes* mosquito (IAM) species. In 2013 a collaboration agreement was signed between the Ministry of Health of the Government of Spain and the Government of the CI to carry out entomological surveillance activities for IAM in the archipelago. The Institute of Tropical Diseases and Public Health of the CI is responsible for carrying out entomological surveillance at the points of entry of the islands. This consists, on the one hand, of active surveillance through sampling at ports, airports and companies involved in importing plants. Samples are collected every 10 days and analysed and identified morphologically (larvae and adults) and molecularly (eggs). On the other hand, citizen collaboration is promoted through the Canary Island Health Service through bites notification provided to primary care centres and pharmacies. In the ten years that entomological surveillance has been active in the archipelago, there have been five episodes of detections of IAM. The first took place in 2017 on the island of Fuerteventura where the entry of *Ae. aegypti* was detected thanks to citizen collaboration. Due to the rapid actions of the competent entities, it was declared eradicated in June 2019. In march 2022, as a result of active surveillance, larvae were detected in an ovitrap in the port of Santa Cruz de La Palma, morphologically and molecularly confirmed as *Ae. aegypti*. Since no new specimens of this mosquito were detected in the last 18 months, this episode is considered to be ended in September 2023. The last three episodes of *Ae. aegypti* introduction have taken place on Tenerife Island. The first was based on a citizen's notification of the presence of adults in a private house, in December 2022. The second episode is based on active surveillance and consists in punctual detections, in January, February, March and May 2023, of adults of *Ae. aegypti* in a BG-Sentinel trap located at the cruise terminal of the capital of Tenerife. The latest episode is the first detection of *Ae. albopictus* in Tenerife and in the CI. It occurred when adult specimens were detected in a greenhouse associated with import of plants. Tenerife episodes are still active but no further specimens have been detected to date, which shows that early detection and control measures are essential to prevent the establishment of invasive aedines.

Session 2. Best practices for mosquito control and biocide management

ORAL PRESENTATIONS

Delaying the establishment of *Aedes albopictus*: mosquito foci elimination in private places in the Netherlands

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In the Netherlands, besides their detection at points of entry, incursions of *Aedes albopictus* have been notified in private places since 2016 by citizens. The policy of the Netherlands is to prevent the establishment of *Aedes* invasive mosquitoes as long as possible. We here describe and evaluate the actions taken since 2016 during *Ae. albopictus* foci elimination campaigns in order to serve as an example for best practices for mosquito control in urban areas. Citizen notifications with pictures informing of the presence of *Ae. albopictus* are received by the NVWA service with an online filled form. After evaluation, if *Ae. albopictus* is confirmed in field samples taken by inspectors at suspected locations, door-to-door intensive inspections are initiated within an area of at least 100 metre radius from the finding locations. In this area, all present potential larval habitats for container-breeding *Aedes* are sampled and subsequently removed, or treated with larvicide (Vectomax FG). A second area is defined from the limits of the 100 metre area until 500 metre radius from the *Ae. albopictus* finding location. Within this area, only public potential larval habitats (mostly street catch basins and storm drains) are treated with larvicide. Additional to the larval samplings, mosquito traps are deployed in the area to monitor the presence of the species during the season. From 2021, GIS-based mobile phone applications were used to record data of the field operations in all private places included in the actions. Between 2016 and 2022, introductions with confirmed introduced specimens of *Ae. albopictus* were notified by citizens in 20 municipalities. Immature stages were found breeding in artificial containers in the backyards of private houses in 15 municipalities, and mosquito control was initiated. Entomological surveillance showed that all detected foci were successfully confined, containing the spread of the species to surrounding urban areas. Also, the majority of the detected foci were eliminated in one or two seasons. Lessons learned since 2016 are: 1. *Ae. albopictus* introductions related to ground traffic (hitchhiking in vehicles from colonised areas) are a fact, and such introductions became more frequent in the last years; 2. the species can easily proliferate during the summer months; 3. prompt citizen communication on the presence of the species is crucial for effectively perform foci elimination; 4. GIS-based mobile application is a key tool to follow and evaluate field operations; and 5. rapid mosquito control is crucial to prevent (or delay) the establishment of *Ae. albopictus* as long as possible.

The mortality of *Aedes albopictus* larvae in natural breeding sites

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Larviciding is the most effective methods to fight against mosquitoes but larvicides have to be tested to ensure their efficacy, before to be put in the market. When the test is performed in laboratory all the parameters can be controlled but the results can be very far from the real conditions, while field test or simulated use conditions are much more realistic. When a test is performed it is necessary to include the control replicates but when a laboratory test is performed the mortality in the control replicates can be reduced near to 0% while when a simulated test is done, the mortality in the untreated replicates can be very high. This is much true closer to the natural conditions we go. Especially for products with a long term efficacy, when the water is left in the buckets for months, in presence of mud or organic matter, algae and bacteria naturally develop causing a very high mortality. In these conditions the larval mortality in control replicates has to be ignored and the efficacy of the larvicide should be calculated just as difference of emerging adults respect to the control. But how is the larval mortality in the real or in simulated natural conditions? A very few studies are available but it seems it can be very high. The study was run placing 40 buckets containing 22 liters of water in the field, leaving them uncovered to allow leaves and insects to fall into the buckets as well as the rain to fill up the buckets. Five months later, 30 second instar larvae have been placed into each bucket. Every day during the next weeks, the adults developed were collected and counted, allowing to define the natural mortality of the larvae. Two months later a new introduction of larvae was done. The larvae introduced into the buckets were *Aedes albopictus* obtained from eggs collected in the field, using ovitraps. Water parameters (temperature, pH, TDS, EC, salinity) have been registered. The larval natural mortality was above 20%, therefore higher to the normal value accepted by several authorities and indicated in some guidelines, making clear the need to ignore such parameter focusing just on the reduction of the emerging adults. This indication can be extended in general to other parameters indicated in guidelines or official procedures which are established for laboratory conditions very far from the real ones, or arbitrarily defined. These parameters, very difficult or impossible to respect, cause the rejection of good test performed in real or simulated conditions.

First characterisation of knock-down-resistance mutations within the *vgsc* gene in *Culex pipiens*

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The only insecticides allowed in European Union for adult mosquito control are pyrethroids, targeting the voltage-gated-sodium-channel (VGSC). A concerning rise in resistance to pyrethroids (PR) in the last decade is threatening their effectiveness. Despite recent reports of phenotypic resistance from Greece, Spain and Italy, little is known on the genetic bases of PR in *Culex pipiens*, the most abundant nuisance mosquito species in Europe and main vector of West Nile virus. The aim of the present study was to provide a first overview on knock-down-resistance (*kdr*) mutations within the *vgsc* gene possibly involved in PR mechanisms in European *Cx. pipiens* populations. An oligo-hybridisation capture approach was performed to allow high-coverage sequencing of the whole vgsc gene for 82 *Cx. pipiens* specimens sampled in Italian and Greece. Alignment, variant filtering and calling of obtained reads was performed using the MoNaS pipeline. Overall, 659 variants were identified across the whole *vgsc* gene. Missense mutations with known or suspected impact on PR were detected for 5 amino acid positions: 1. Locus 1014: 1014F allele was found in all sampled regions with an allelic frequency varying from 25 to 87%, while 1014C allele was found only in Greece (freq=50%) and 1014S was found in a single north-Italian specimen in heterozygosis; 2. mutation F1534L was present in all Italian regions at an overall frequency of 8% but absent in Greece; 3. mutations at frequencies <5% were detected in positions 253, 1879 with mutation M918T being detected for the first time in mosquitoes. Obtained results depict a worrisome picture with widespread *kdr* mutations in Italian and Greek *Cx. pipiens* populations and provide data for future investigations on the impact of novel mutations and their possible synergism, as well as for the development of diagnostic tools for *kdr* variants. Such data are crucial for the study of resistance mechanisms and for the choice of the most appropriate insecticide resistance management strategies aiming at maintaining the effectiveness of the only chemical tool nowadays available for the reduction of adult mosquito abundances.

Behavioural resistance in malaria vectors occurred prior to the scale-up in insecticide-based vector control

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The decline in malaria prevalence since the year 2000 has been attributed primarily to the upscaling in access and use of insecticide-treated nets (ITNs) and indoor residual spraying (IRS). However, malaria cases have been declining for over 100 years, despite limited programmatic vector control during most of this time. This study investigated if changes in dominant vector behaviours might explain reductions in malaria morbidity and mortality. A comprehensive georeferenced dataset of all dominant vector behavioural data extracted from 875 manuscripts published from 1985–2011 (>10 000 data records) across Africa, the Americas, and the Asia-Pacific region was analysed. The proportion of blood meals on humans and the proportion of bites indoors by *Anopheles gambiae* s.s., *Anopheles arabiensis* and *Anopheles funestus* s.s. diminished from 1985 to 2011, thus lowering the vectorial capacity of these three dominant African malaria vectors. Such behaviour shifts define behavioural resistance to insecticide-based interventions in which vectors avoid contact with the insecticides in ITNs and IRS. However, the behavioural changes reported here started before the onset of the scale-up in ITNs and IRS in the early 2000s, suggesting that other widespread drivers were exerting pressure to prevent vector house entry and feeding on humans in an analogous manner to ITNs and IRS across Africa, such as house improvements and untreated bed nets. To investigate if these unexpected findings were a function of bias in how the data was collected, the dataset was analysed for changes in the mosquito sampling methods used and variations in the geographic origin over the 26 years of data. Evidence in sampling method bias was not found, as the methods used to collect malaria vectors in Africa were consistent from 1985 to 2011. Human landing collections (HLCs) indoors, outdoors or conducted simultaneously indoors and outdoors were the dominant collection methods used to study the HBR and the indoor biting proportion, while indoor and outdoor resting collections most often were used to determine the HBI. Additionally, the geographic origin of the data did not change significantly in the reported period. These results demonstrate the power of entomological surveillance to enable early detection of behavioural shifts that may threaten the effectiveness of malaria vector control. The findings of reduced house entry and diminished human feeding provides an explanation for the declines in malaria prevalence that occurred prior to the scaleup in ITNs and IRS beginning in 2004.

Session 2. Best practices for mosquito control and biocide management

POSTERS

Effect of gender in the duration of the topical repellent efficacy against mosquitoes

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Topical repellents are an important category of products and are widely used. The efficacy of topical repellent is normally evaluated using a protocol defined as 'arm in cage' where a volunteer introduces at time intervals a treated arm into a cage where starved mosquitos have been released. The different parameters are: Species of mosquito, strain, size of the cage, number of mosquitoes, age of the mosquitoes, time of counting, frequency of counting. The efficacy of the repellent is defined by the 'complete protection time' (CPT) which is 'the time from the application of a repellent until the last effective observation, before the efficacy failure by a confirmed event. The CPT to be specified corresponds to the time interval before the confirmed event' (ECHA guidelines 5.0). Several Guidelines indicate to use volunteers of both genders for testing the efficacy of repellents, but is really there a difference in the attractiveness between males and females? Does the gender really affect the efficacy of a topical repellent? The effect of the gender when performing efficacy evaluation tests is an important topic because it affects the choice of the participants and the reliability of the results. Data were gathered from efficacy tests of commercial topical repellents performed along years. The tests were performed following the method Arm in cage but many parameters were not constant. Some parameters were changed depending of the product and of its commercial claim, like species (mosquitoes: *Ae. albopictus*, *Ae. aegypti*, *An. gambiae*, *Cx pipiens*, *Cx quinquefasciatus*; tick: *Ixodes ricinus* nimphs) active ingredient AI (Icaridin, Citrepel, IR3535, PMD, Citrodiol, DEET), concentration of AI, formulation (lotion, aerosol, wipe, roll-on, gel) and dosage. The analysis of the effect of the gender on the CPT duration was done comparing the CPT values registered within a Test, where for Test it is intended a number of replicates performed by different volunteers, applying the same parameters (product, dosage, length of the assessments, frequency of the assessments, species of mosquito, number of mosquitoes). The effect of the gender on CPT was measured analysing 414 replicates from 38 Tests. The analysis of the data clearly demonstrated that the gender of the volunteer does not affect the protection provided by topical repellents.

Efficacy and repellence of a Lethal Ovitrap coated with Insefly 5A IGR NG: laboratory tests

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Lethal Ovitraps are a mosquito control method in which gravid females are attracted to a container where they are affected by biocides (larvicides and/or larvicides). The Insefly 5A IGR NG paint contains Alpha-cypermethrin (0.7%) and D-Allethrin (1.0%) as adulticides, as well as the larvicide Pyriproxyfen (0.063%). The interior of Anticimex ovitraps were painted with this product (dyed with black), transforming them into Anticimex Lethal Ovitraps. Different aspects of these Lethal Ovitraps have been tested in the laboratory, such as the direct mortality caused to adults and larvae, the viability of deposited eggs, the repellency caused by the trap and the ability to affect other nearby breeding sites. The results have revealed the rapid mortality caused to adults and fourth instar larvae, in less than 15 minutes. The eggs submerged in water hatched normaly, but the larvae died in a brief period of time. No repellence caused by the Lethal Ovitraps was observed, since the females normally entered them. Mortality of larvae has been recorded in containers placed at 60 cm. Despite the effectiveness achieved by these Lethal Ovitraps, different aspects such as mortality caused to non-target species must be investigated before making a routine use of this control method.

Spatial distribution of pyrethroid resistance-associated mutations in *Aedes albopictus* across Southern France

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Controlling the transmission of *Aedes*-borne viruses heavily relies on the use of insecticides, but although the first signs of pyrethroids resistance have been detected in European populations, research on French populations is still limited. In this study, we present a comprehensive investigation into the spatial distribution of knockdown resistance (*kdr*) mutations associated with pyrethroid resistance in *Ae. albopictus* across Southern France using a two-step approach based on multiplexed amplicon sequencing. Initial pooled mosquito DNA sequencing efficiently screened for *kdr* mutations in multiple sites across a broad study area at low cost. Subsequent validation through individual mosquito DNA sequencing validated these findings while giving access to *kdr* allele prevalences and genotypes. Our approach enabled the rapid and cost-effective identification of *kdr* mutations previously linked to resistance by utilising high-throughput molecular assays as surrogates to traditional phenotypic resistance assessments. Our analysis revealed the presence of the V1016G allele in 14 distinct sites. Notably, this allele exhibited a concentrated distribution in the South-Eastern part of France, particularly in proximity to the Italian border. Additionally, isolated occurrences of the V1016G allele were detected near Bordeaux and Marmande. Remarkably, all identified mosquitoes carrying the V1016G mutation were found to be heterozygous, suggesting a lack of phenotypic resistance to pyrethroid insecticides. Furthermore, our investigation identified four additional mutations within our targeted genomic sequence: I1532T, M1006L, M1586L and M995L. Through the sequencing of a segment of the maternally inherited mitochondrial genome, we confirmed that the colonisation of *Ae. albopictus* in France originated from founders exhibiting haplogroup A1. Overall, these findings contribute valuable insights into the resistance dynamics of *Ae. albopictus* populations across Europe. By delineating the spatial distribution of pyrethroid resistance-associated mutations, we enhance our understanding of challenges posed by resistance to effective vector control.

Lack of pyrethroid resistance alleles in *Aedes caspius* sympatric with resistant *Ae. albopictus* and *Culex pipiens*

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Mosquitoes are not only a relevant nuisance, but also important pests causing damage to human and animal health and are therefore target of extensive pesticide treatments. This has caused development and spreading of resistance to most largely used insecticide molecules in many species. In the European Union, only pyrethroids – targeting the voltage-gated-sodium-channel (VGSC) – are allowed for the control of adult mosquitoes and for the limitation of ongoing disease transmission, but their excessive usage has led to the rise of pyrethroid resistance (PR) in both the most widespread species, *Aedes albopictus* and *Culex pipiens*. Herein, we focus on *Aedes caspius*, a floodwater species whose marked anthropophily and aggressive biting behaviours strongly affect outdoor activities, and which is a major target of mosquito control programmes in Italian touristic areas. Despite this no data are available concerning its PR status. *Aedes caspius* specimens were sampled in 2020 in three coastal sites from Ferrara province in Italy were previous studies highlighted high phenotypic PR and high frequencies of knock-down-resistance (*kdr*) mutations within the *vgsc* gene associated with PR in sympatric *Ae. albopictus* and *Cx. pipiens*. Sequencing of domains I, II and III of the *vgsc* was carried out to investigate the presence of mutations with known impact on PR in mosquitos. DNA was extracted from 121 specimens and sequencing was successful for 43, 101 and 61 specimens for domain I, II and III respectively. No known kdr mutations were found in the examined specimens. Despite the reported high insecticidal usage in the sampling sites and the high presence of phenotypic and genotypic PR in sympatric *Cx. pipiens* and *Ae. albopictus* (where frequencies of *kdr* alleles were above 90% and 25%, respectively), the present study did not detect any *kdr* mutations in the *Ae. caspius* sample analysed. This might be due to actual full susceptibility of the analysed populations to pyrethroids, which will be evaluated soon by bioassays. On the other hand, the species may rely on metabolic or cuticular resistance mechanisms as an alternative to the target-site resistance mechanisms investigated herein. Further investigations are ongoing to clarify these aspects and to understand the possible differential selective pressure of pyrethroid usage on different mosquito species.

Session 3. Vector control in the context of disease outbreaks

ORAL PRESENTATIONS

ARBOPREVENT: Moving from reactive to proactive strategies for the prevention of West Nile virus in Spain

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In Europe, West Nile virus (WNV) has reemerged in the last decades. Human cases were scarce in Spain until summer 2020. Since then, 83 cases of severe infection and 10 fatal cases have been reported, most of them in Andalusia (South Spain). ARBOPREVENT is a 3-year project (2023–2025), funded by Fundación 'la Caixa', focused on providing the scientific basis to identify the risk areas of WNV in Andalusia based on the identification, distribution, abundance, trophic and environmental preferences of the main mosquito species involved in WNV transmission. At the moment, we have sampled ca. 500 sampling points (we expect to sample 1000) across the main land cover categories in three provinces of Andalusia (Seville, Huelva, and Cadiz), where the circulation of the virus has been documented. So far, ca. 30.000 females of 21 mosquito species (1/3 of the total Spanish species composition) were trapped by BG-Sentinel traps supplemented with CO_2. Potential vectors of WNV (*Culex pipiens* and *Culex perexiguus*) are widely distributed in western Andalusia and accounted for half of the total mosquito collections. The presence of mosquitoes was reported in 92% of the sampling points. Out of the 1059 mosquito pools analised, 30 pools (4 of *Cx. pipiens* and 26 of *Cx. perexiguus*) were positive to WNV by RT-qPCR assays in different munipalities. Characterisation of mosquito diets (n=300) by blood meal analyses is in progress. Real time information on mosquito captures at key localities is provided at the website http://mosquitos.ebd.csic.es together with basic ecological information on the mosquito species present in Spain and its public health relevance. All this information is allowing regional health and local authorities to adaptatively manage their mosquito control programmes. At the end of the project, we expect to design an early warning system to predict both the risk of mosquito proliferation and WNV spillover into humans.

Human-mosquito biting networks and the dynamics of mosquito-borne diseases

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As scientists and public health authorities around the world struggle to react to the re-emergence and increasing incidence of mosquito-borne diseases, we lack critical information about the interactions between vector mosquitoes and their human hosts, and about the resulting networks through which diseases flow. Traditional epidemiological models assume homogeneous biting patterns, yet this assumption has been called into question by field studies. If biting patterns are heterogeneous, traditional models may be underestimating disease risks and providing poor guidance for mosquito control decisions, but we do not have a strong basis for correcting these models. This presentation provides a starting point for addressing the problem. The work draws on a new source of high resolution mosquito exposure estimates in Barcelona, Spain, based on the Mosquito Alert citizen science platform, AI-driven smart traps, and traditional mosquito surveillance. It uses this to make inferences about the range of possible degree distributions in human-mosquito biting networks. It then uses agent based models to explore the epidemiological consequences of these distributions. It compares the basic reproduction number and attack rate for simulated outbreaks using the degree distributions estimated from the data, holding constant the number of humans, mosquitoes, and mosquito bites (network links) in each simulation. In other words, each simulation takes the same number of total humans, mosquitoes, and mosquito bites and simply rearranges the bites based on the variation in real-world mosquito exposure risks estimated in Barcelona. This produces different distributions in the number of different mosquitoes that bite each person and the number of different people each mosquito bites. The goal here is not to estimate actual epidemic risk, but to see how small differences in biting networks can influence epidemic outcomes. The results show that these small differences can have important influences. For example, the mean basic reproduction numbers in the Barcelona simulations range from under 2.4 to 3.0, with standard deviations showing very clear separation among the different degree configurations. These results provide insights into the extent to which homogeneous mixing models of disease dynamics give misleading risk estimates, and how this can play out across geographic space, providing a starting point for improving estimates of mosquito-borne disease risk and strengthening prevention, control, and treatment strategies.

Effects of sublethal doses of insecticide on egg-laying, biting and resistance of *Anopheles gambiae*

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The extensive use of insecticides for vector control and agriculture has led to environments rich in these compounds, namely water bodies, which mosquitoes depend on to complete their life cycle. Continuous exposure to sublethal doses of insecticide has long-term consequences for several aspects of the life-histories of mosquitoes, but little is known about its impact on their biting motivation and egg-laying preference, which are important parameters for their population dynamics and thus for the epidemiology of infectious diseases. Using an insecticide-sensitive and a resistant population of the main malaria vector *Anopheles gambiae*, we assessed how exposure to a sublethal dose of the permethrin insecticide throughout larval stage affects the egg-laying preference, biting motivation and sensibility of adults. We measured these egg-laying preference by giving females the choice to lay eggs in a container with water supplemented or not with permethrin. We measured biting motivation in a two-way choice situation by letting them choose between blood-feeding through an insecticide-treated or an untreated net and measuring the proportion having chosen the treated net. We also measured this in a one-way situation by letting them choose between feeding either through an insecticide-treated or through an untreated net or not feed at all, and measuring the proportion having fed on each of the two nets. All blood-fed mosquitoes were kept to measure their blood-meal size, assessing the possible cost of biting through impregnated nets. We measured sensitivity to insecticide according to the WHO protocol, counting knockdowns during an exposure of 30 or 60 minutes and mortality 24 hours after exposure. Overall, our results showed that exposure to insecticide throughout larval stage led to adults avoiding exposure to the insecticide less strongly during their search, both for blood sources and egg-laying opportunities. However, it did not change the sensitivity of the adults to the insecticide. These results raise important concerns about the use of insecticides for vector-borne diseases and pest control and reinforce the need for new vector and pest control strategies.

A hybrid model to unveil the role of *Culex torrentium* in West Nile virus transmission in Europe

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Driven by globalisation and climate change, mosquito-borne viruses have emerged in Central Europe over the last decades, with Usutu virus transmission detected in Germany in 2010, and West Nile virus (WNV) in 2018. Although *Culex pipiens* is regarded as the main vector, the morphologically similar species *Culex torrentium* has been shown to yield higher transmission efficiency rates for WNV. Thus, *Cx. torrentium* may play a significant role in WNV transmission, especially in Northern and Central Europe, where it is found as a common or even predominant species, often occurring in sympatry with *Cx. pipiens*. Mechanistic and correlative modelling approaches are used to evaluate the risk of WNV transmission, but there is a gap in literature that integrates both streams for more informative results. Moreover, most models employ static data instead of regularly updated data, limiting their ability to make near-real time predictions. In this study, a 'hybrid' model was developed in which estimates of mosquito abundance were used to refine two distinct mechanistic R_0 models based on temperature-dependent and taxa-specific transmission parameters. Mosquito abundance was estimated from real-time surveillance data collected from traps across Germany, and allowed deriving the vector-to-host ratio parameter fed into the models. Nation-wide climate data updated on a daily basis served as model input to yield short-term forecasts presented as risk maps, which can be easily interpreted and used as a tool for risk assessment. Results suggest that the role of *Cx. torrentium* in WNV transmission may have been grossly underestimated, as its high vector competence for this virus generated high R_0 values. Experimentally-derived data for the WNV transmission efficiency at 21 days post-infection resulted in a higher R_0 for *Cx. torrentium* than *Cx. pipiens* (up to 23 and 17, respectively). A second R_0 model based on a relative metric of temperature suitability showed that the transmission optimum for WNV occurs at 24°C for both *Culex* species. In the light of climate change, WNV circulation is predicted to rise in northernmost areas of Europe where climate is currently temperate and *Cx. torrentium* is the predominant *Culex* species. Therefore, integrative models that take advantage of regularly updated climate and mosquito monitoring data could be a valuable asset for decision-makers, especially in what pertains to surveillance measures to enable early detection and subsequent interventions.

Epic 2023 rainfall causes mosquito population explosions and impacts West Nile Virus surveillance in Colorado

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The front range of Colorado has a population of nearly 5 million people spread out over a mosaic of urban/suburban build-up and intensive irrigation-based agriculture. Like many European countries, the temperate climate and harsh winters produce a 15-week summer 'mosquito season' during which time mosquito surveillance and control is practiced. Populations of vector species in the genus *Culex*, especially *Cx. tarsalis* are driven by extensive cattail marshes associated with seepage from earthen damns and other human-generated and natural standing waters. *Cx. tarsalis* is the main vector of endemic West Nile Virus (WNV) here. Our purpose is to assess the impacts of record drought-busting precipitation on large-scale mosquito surveillance conducted by seasonal employees from regional University science programmes. Seasonal mosquito surveillance staff for Vector Disease Control International (VDCI, a Rentokil Company) were specifically recruited from the Biology Department at Metropolitan State University of Denver and other regional Universities. Surveillance technicians set CO_2 baited CDC mini light traps, retrieved them the next day and returned to the lab for sorting and pooling of *Cx. tarsalis* into groups of 65 which were then tested for WNV. We selected 5 traps within each of 3 focus zones: Urban (U), Suburban (SU) and Rural (R) and compared monthly rainfall and mosquito abundance between 2022, a typical year, and 2023, the record-breaking year. Cumulative rainfall in each of the 3 focus zones during May and June did not exceed 10.5 cm while totals during 2023 were: U=29 cm, SU=28.2, R=26.8 cm. In all zones, the mean numbers of *Cx. tarsalis* per trap in June/July 2023 were dramatically higher than in 2022: SU=135.5+35.9 (2022), 766.2+300.8 (2023); R=70.6+13.8 (2022), 652.7+117.5 (2023); U=8.8+2.2 (2022), 95.7+24.6 (2023). Through July of 2023, 20.9% (N=182) of *Cx. tarsalis* pools tested from our selected zones for WNV have been positive. Due to high numbers of trapped mosquitoes overtime was necessary in order for surveillance staff to complete trap processing and pooling for WNV: surveillance staff consistently worked 10–20 hours overtime per week. Through all the staff were proud and maintained high morale. In conclusion mosquito surveillance data collected by this thorough and expansive approach have been critical to the successful surveillance of WNV. By specifically recruiting undergraduate students of the biological sciences we are filling seasonal staff positions with talented, flexible and driven employees who view their jobs as a career step.

Management of major mosquito species, with special emphasis on vectors of dengue and malaria in Pakistan

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In Pakistan major mosquito-borne diseases (MBDs) are malaria, dengue and chikungunya, of which dengue is the fastest emerging arboviral infections since 2005. During 1995–2004, only 699 dengue confirmed cases and 6 deaths were reported from 3 districts. These numbers dramatically increased to 238 985 and 1990, respectively, affecting 115 districts during 2005–2022. 91.8% of total cases were reported from 9 mega cities having 4000–5500 population/km^2. *Aedes aegypti* and *Ae. albopictus* are major vectors species and have been associated with man-made clean water domestic habitats. During 2010–2021 annually 300 000 to 375 000 confirmed malaria cases were report from country. Due to massive floods in 2022, 3.0 million cases were reported. *Anopheles culicifacies* and *An. stephensi* are considered major malaria vectors in Pakistan. IRS and ITNs are major interventions. For malaria control coverage levels of IRS and ITNs are 30% and 100%, respectively. Resistance development, low coverage through IRS and low rate of utilisation of ITNs (34%) are the key challenges for programme. For dengue mosquito control community-based Larval Source Management (LSM) is key intervention. As a part of national insecticides resistance management strategy, *Bacillus thuringiensis israelensis* and Spinosad were evaluated at different concentrations against 3rd and 4th larval instars of *Ae. aegypti* and *Ae. albopictus*, *Anopheles culicifacies*, and *A. stephensi* since 2020. Concentrations for *Bti* were 0.2, 0.4; 0.8; and 1.0 mg/l equivalent to surface application 0.2 to 1.0 kg/ha. On day 0, 7, 14 and 21, 150 ml were applied. All used concentrations successfully killed 100% larvae of *Ae. aegypti*, *Ae. albopictus*, *An. culicifacies*, and *An. stephensi* within 24 hours of application both in field and laboratory conditions, while higher concentrations showed slightly prolonged residual effects. Early instar larvae were found after two days in concentration of 0.2 and 0.4 mg/l, and after three days in the higher concentrations. On days 6 and 7 after application, all concentrations showed late instar larvae. However, pupae in small number were observed on days 25 and 26 in field and laboratory conditions, respectively. Pupal reduction in laboratory and field was 98% and 99%, respectively. A slight variation in larval densities were noted in different species particularly in filed applications. Spinosad also yielded significant levels of mortalities ranging 97–99% of different mosquito species during target period. Based on significant results country is planning to scale up the use of biorational insecticides for sustainable management of MBDs.

Session 3. Vector control in the context of disease outbreaks

POSTERS

A mathematical model for the transmission dynamics of Usutu virus in Germany

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Usutu virus (USUV) has emerged as a significant public health concern in Europe, including Germany, showing a rapid spread and impact on avian populations. In this study, we proposed a deterministic mathematical model to characterise the transmission dynamics of USUV between *Culex* mosquitoes and European blackbirds. Our model incorporates essential factors such as mosquito population dynamics, driven by climate variables like temperature, rainfall, and wind speed. We analysed the model using mathematical techniques to gain insights into the dynamics of USUV transmission. Through sensitivity analysis, we investigated the influence of key factors, such as the mosquito offspring number and the basic reproduction number, on the spread of the virus. Results from this analysis showed that reducing the number of eggs laid by each female mosquito can reduce the density of mosquitoes, hence leading to a decrease in the spread of USUV. We introduced male *Wolbachia* mosquitoes as a control measure for reducing the number of eggs laid by a female mosquito, and the intraspecific competition for females and resources necessary for survival between *Wolbachia* infected males and wild males is studied. Additionally, we extended the model to include spatial distribution, which takes into account movement of birds and mosquitoes at different locations. Results from the spatio-temporal model show that the spatial distribution of mosquitoes is influenced by the location of the host, while the temporal distribution is influenced by climatic conditions. By integrating epidemiological, ecological, and environmental factors, our mathematical model offers a comprehensive understanding of USUV transmission dynamics between mosquitoes and birds in Germany at a spatio-temporal level. Insights derived from this study can guide surveillance strategies, inform evidence-based public health policies, and aid in implementing targeted interventions to mitigate the impact of USUV on avian populations in Germany. In conclusion, this research provides a valuable tool for decision-makers to develop proactive strategies for the prevention and control of USUV, ultimately protecting public health and preserving the well-being of avian populations in Germany.

Carry-over and fitness effects in *Culex* species along a land-use gradient

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In the current scenario of climate warming, the outbreak risk of mosquito-borne diseases such as West Nile virus (WNV) will increase in central Europe. To properly assess the vectorial capacity of *Culex* species and effectively design control and monitoring plans, it is essential to understand the underlying factors which determine population development. Therefore, field and laboratory experiments were carried out between 2020–2022 to analyse how the environmental conditions of the breeding habitats affect the fitness of emerging adults (so called carry-over effects). In a field study, insect cages with artificial breeding habitats containing first instar larvae of *Culex torrentium* were installed along a land-use gradient from vegetation-dominated areas to urban settlements, and regularly checked for adult emergence. A portion of those adults were taken to the lab, where they were tested for longevity (as a proxy for fitness) under thermic stress conditions. The rest of the adults were taken to the lab and wings were dissected and measured. Results showed that the highest survival probability to the adult stage occurred between 20–21°C, and larval developmental time decreased with higher temperatures in urban sites, while mortality increased. Females from vegetation-dominated and suburban areas also had a higher longevity than those from urban areas, while there were no significant differences among males. Simultaneously, adults from urban sites with higher maximum temperatures also had a decreased wing centroid size, possibly indicating decreased fecundity. Wing asymmetry increased with higher maximum temperatures, which might be indicative of environmental stress. These results provide new insights into the microhabitat-dependent ecology of *Culex* species and can refine our understanding of the spatio-temporal development of *Culex* populations.

The impact of various parameters of water bodies for the abundance and species composition of mosquito larvae

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Mosquitoes are considered to be the most dangerous organisms because of their ability to transmit a huge variety of pathogens and cause outbreaks of various diseases. Mosquitoes are able to inhabit vast territories due to a wide range of mosquito larval habitats. The knowledge of mosquitoes' choice for egg-laying, conditions of larval habitats, and spatial distribution is very important for vector control strategies. In this study, mosquito larvae and pupae were collected in order to observe species diversity and seasonality in various water bodies and the relationship between some parameters and the presence or absence and abundance of mosquito larvae in investigated water bodies. Mosquito larvae (5392) were collected in 2021 (March-October) and in 2022 (April-July) in various water bodies in Lithuania. Water temperature (°C), pH value, amount of NO_2 (mg/l) and NO_3 (mg/l) were measured during the sampling. The size of the water body, bottom coverage, exposure to sun, and temporality were also observed. During this study 25 mosquito species have been detected, species diversity, seasonality and the impact of investigated parameters on the larval abundance of certain mosquito species were evaluated. Both multiple regression and redundancy analyses showed the temporality of water bodies and the time of the year are important parameters for the larval abundance of some mosquito species in water bodies. The amount of NO_3, pH values and bottom coverage were also important for the abundance of larvae of some mosquito species. This research will help to understand the local mosquito distribution and will help to lay the groundwork for the renewal of Lithuanian mosquito fauna research.

Session 4. Citizen science and community involvement for mosquito surveillance and control

ORAL PRESENTATIONS

Next generation community-based surveillance of mosquitoes: coupling citizen scientists, experts and machines

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Monitoring the spread of invasive mosquito species (IMS) requires effective and rapid surveillance over large areas. Since 2014, Mosquito Alert (MA) has demonstrated the value and scalability of real-time data from citizen scientists using mobile apps in contrast to field surveillance, which has coverage and cost challenges. Citizen science increases the detection of new IMS populations, especially when they are found far away from known areas. In MA, each digital report including a picture is validated by 3 experts from a pool of 104 entomologists to assign a species-probability class in a standard manner. Speed of human validation depends on both report input rate and expert availability. The European launch in 2020 increased the participation, with occasional congestion in the pipeline and thus a challenge to real-time surveillance. In addition, MA is now included in the Spanish official surveillance plan and it is increasingly relied on by regional and local PH authorities, requiring a new validation protocol to allow automated alerts in real time. An artificial intelligence system (AIMA), a set of neural networks, has been integrated into the MA workflow to automatically examine each report to calculate a species probability. It sends a preliminary message to the user and sets an alert if the species is not known to occur in the area, which can be routed internally or automatically e-mailed to PH officers or MA national delegates. The maps used to assess the known distribution of each species are public (https://map.mosquitoalert.com). They are produced by merging bi-annual ECDC information with historical records from ReNED network members and real-time MA data. The resolution is NUTS3 in Europe; In Spain, the maps are at the municipal level. AIMA can be given variable levels of autonomy, including direct publication on the system's live public web map. The system retains reliability, as human experts continue to oversee IA outputs, and gains speed, as new positives are detected within minutes. In this pilot period AIMA's precision is species-dependent and up to 0.84 for *Aedes albopictus*. As a result of this new combined system, *Ae. albopictus* presence has been detected for the first time in 51 municipalities in Spain, including a whole new region.

Overwintering of tiger mosquitoes (*Aedes albopictus*) in two villages in Belgium

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Aedes albopictus is an important invasive species due to its potential as a vector for viruses, such as dengue, chikungunya and Zika. Once the species is established in a region, the chance of local transmission of these viruses significantly increases. Therefore monitoring of the tiger mosquito and implementation of control measures is crucial to delay its establishment and reduce the risk of local virus transmission. In 2022 in Belgium active (on parking lots along the highway) and passive (citizens notify tiger mosquitoes) surveillance led to the detection of tiger mosquitoes in 12 locations. Field inspections uncovered multiple tiger mosquitoes in two locations. Control measures were implemented. In order to determine whether *Ae. albopictus* overwintered in Belgium, several actions were undertaken. A sensitisation campaign was conducted, involving information sessions and the distribution of letters, to enhance the participation of the citizens in the monitoring and to encourage them to take preventive measures. Subsequently, larval inspections were carried out at the end of April within the same 200 m buffer zone of last years' findings, followed by the placement of 20 oviposition traps in May, extending to a 500 m buffer zone. The info sessions to sensitise citizens yielded moderate success. The local governments actively participated in disseminating information and sensitizing the citizens about the tiger mosquito monitoring and possible preventive measures. The first larval samplings didn't detect overwintering tiger mosquitoes and no eggs were found in May/June. In mid-July, citizens reported two sightings of a tiger mosquito - one from each location - and oviposition traps at both locations collected eggs. As the notifications and positive oviposition traps were located in the 200 m buffer zone of last year's findings, we conclude that overwintering took place. This is the first evidence of overwintering *Ae. albopictus* in Belgium. The tiger mosquitoes were detected by citizens and eggs in oviposition traps confirmed their reproduction. This marks the transition into a novel phase of the invasion process of the tiger mosquito, characterised by its local establishment. The collaborations with the local governments and the sensitisation and involvement of the citizens will need to be strengthened in the coming years to reduce the populations to a minimum in order to minimise the risk for public health.

Mosquito Alert ITALIA 2020–2022: citizen engagement, achievements and criticisms

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The Mosquito Alert citizen science system has been in operation since 2014. Initially focused in Spain it undertook a major expansion in the rest of Europe beginning in October 2020, with the main goals to: 1. monitor the spread of *Aedes* invasive species and *Culex* sp. (transmitting global arboviroses) through georeferenced, expert-validated photos of adult mosquitoes; 2. assess mosquito biting activity through records of biting; 3. provide support for advanced mosquito surveillance; 4. increase the awareness of citizens about public health problems related to mosquitoes. We present the results obtained by implementation of Mosquito Alert in Italy from its release to end of 2022. Methods. Promotion activities were carried out at national level by press releases, newspaper and TV/Radio interviews, and at local level by University lectures, dedicated seminars, participations to public events. Data on citizen engagement and records were analysed. Results. A total of 18,323 citizens downloaded the App and turned on the sampling effort in Italy (approx.. 40% of overall users in Europe) and approx. 30% of these sent at least one record. Among the 8201 mosquito photos received, 5168 were identified by experts: 36.8% as Culex spp., 58.1% as *Aedes albopictus*, 0.5% as either *Ae. koreicus* and/or *Ae. japonicus*, and 4.5% as other autochthonous mosquito species. Most common species were reported from all Italian regions, while *Ae. koreicus* and/or *Ae. japonicus* were reported from northern regions only. Temporal trend of relative frequencies of the two most abundant species reflects their known seasonality; 11 *Ae. albopictus* males were reported in winter months in 6 Italian municipalities. *Aedes albopictus* represented 90% and 52% of the total mosquitoes photographed outdoors (n=1,087) and indoors (n=1,842), respectively. Conclusions. Results show higher citizen engagement with Mosquito Alert in Italy then in other countries and results on the distribution and biology of the target species. The suggestion of an endophagic activity in *Ae. albopictus* may imply a higher contact with humans than that estimated by data obtained by conventional outdoor collection methods.

Mass trapping for *Aedes albopictus* control in residential areas: NESCOTIGER, a large-scale field trial

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Aedes albopictus is established in several European countries, demonstrating a sustained spreading pattern across the continent. This invasive mosquito is a public health threat because of its vectorial competence for arbovirus transmission. Peri-domestic habits of this mosquito greatly diminishes the efficacy of regular control activities, as individuals may breed in private residential areas. Oviposition behaviour can be exploited for targeting adults and immature stages through different types of traps. We aimed to develop an experimental integrated control programme for *Ae. albopictus* in a residential area in Valencia (Eastern Spain) focusing mainly on a mass trapping intervention in private areas through citizen science collaboration. Collaborating residents used oviposition traps belonging to three modes of action in their gardens during the mosquito season. Evaluation was based in adult collection, oviposition sampling and a voluntary survey about perceived mosquito presence. A total of 1028 families participated in the project and 2884 traps were deployed. The area with adult lethal ovitraps showed the lowest adult collections; in addition, residents living in this sector reported the highest satisfaction rates in the survey. Targeting adult *Ae. albopictus* through the mass deployment of lethal oviposition traps appears to be a promising and rational control strategy in residential areas.

Places and times of human-mosquito interaction in urban areas: insights from Barcelona

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The study of human-mosquito interactions is of paramount importance for assessing and reducing both nuisance and vectorial capacity of mosquito species. Here, we quantify the spatio-temporal patterns of human-mosquito interactions by combining complementary sources of data from citizen science, collected by Mosquito Alert, and from active field surveillance. These sources provide estimates of population distribution and biting activity for both the tiger mosquito (*Aedes albopictus*) and the common house mosquito (*Culex pipiens*). They are combined with data on human settlement patterns, activity spaces, and both built and natural environment. We analyse data from October 2020 through December 2022. From Mosquito Alert, the information about nuisance includes 980 bite reports with associated information on the number, location (indoors or outdoors) and time of the day of the bites. Additionally, a total of 330 adult mosquito reports were validated by experts as 'probably' or 'definitely' *Aedes albopictus* (nearly 62% of them) or *Culex pipiens*. From field sampling data, we used the weekly sampling of 30 BG-Sentinel2 traps. Preliminary results suggest that bite reports were heterogeneously distributed across the city, with the largest cluster located around the city centre, while adult reports were more homogeneously distributed. Reports of *Aedes albopictus* concentrated from April to November while *Culex* reports were more homogeneously distributed throughout the seasons. Considering the aggressive daytime biting dynamics of *Aedes albopictus* and the nocturnal preferences of *Culex pipiens*, bite reports were attributed to each species depending on the time of the day declared by each user. The highest biting activity coincided with the highest number of reports of *Aedes albopictus* on the hours before sunset. These results are now being adjusted based on citizen science sampling effort and modelled with socio-demographic and environmental characteristics to make inferences about spatio-temporal patterns and their determinants. Our results indicate the complex human-mosquito interaction patterns in urban areas and the need of fine resolution, complementary sources of data to understand the underlying mechanisms.

Buzz and croak: microwetland biodiversity links through field, experimental, and citizen science studies

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Micro-wetlands are increasingly important figures in the protection of biodiversity. However, sometimes these small ecosystems are seen as a source of nuisance associated with mosquitoes. The MIKROKLIMA project aims to create a micro-wetland monitoring network to improve the biodiversity and climate resilience of the landscape, in the Basque Country. Based on the hypothesis that higher diversity and abundance of amphibians is inversely correlated with the abundance of mosquitoes, the drivers of mosquito abundance and its relationship with pond biodiversity are being studied though three angles: field study, experimental study, and citizens science. The field study was conducted in 25 ponds located in urban and natural areas. Amphibians and mosquito larvae sampling were carried out between April and June 2023. Relationships between of their abundance were evaluated. No significant associations were found using preliminary data. Regarding experimental pilot study, the aim was to determine the interaction of amphibians in mosquito larvae. In June, 12 water containers (200 litre) were placed in a suburban environment: four with 22 *Alytes* tadpoles each, four with 23 *Triturus helveticus* newt larvae and four as control. Ten days later, water was examined, and mosquito larvae were count. All the larvae correspond to *Culex pipiens* s.l.. Larval density was significantly lower in the newt tanks (p=0.02). Finally, the School Ponds Network engaged students and teachers in a mosquito study challenge. They received guidance and protocols to enhance their knowledge of mosquito biology, ecology, and importance. Students collected mosquito larvae samples from their ponds, observed their metamorphosis into breeders, and sent adult mosquitoes to NEIKER for species identification. A total of 13 educational centres participated in the challenge and overall, 67.5 litres of water were evaluated. Six different mosquito species were identified. However, inability of certain students and teachers to distinguish between Chironomidae and Culicidae mosquitoes was detected. Understanding the biological interactions in these ecosystems is fundamental to developing appropriate actions, such as promoting the naturalisation of urban ponds. Equally crucial is familiarising citizens with the biodiversity of these ecosystems, fostering a deeper connection to their preservation and avoiding unrealistic negative perceptions.

Session 4. Citizen science and community involvement for mosquito surveillance and control

POSTERS

Mosquito nuisance and control in the United Kingdom in 2022: a questionnaire-based survey of local authorities

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Mosquitoes are widely distributed across the United Kingdom, and changes in their distribution, particularly those linked to nuisance biting, are expected to increase queries to Local Authority (LA) Environmental Health teams (EHO). The Mosquito Nuisance and Control Survey aims to record these changes and shed light on mosquito nuisance biting incidents and control practices. The questionnaire has been conducted in the United Kingdom (UK) since 1970, with the most recent survey conducted in 2022 by UKHSA (United Kingdom Health Security Agency). The questionnaire was distributed to EHOs within all 373 local authorities across the UK. Participants were given the option to complete the questionnaire online, via email, or by post to ensure comprehensive data collection. The survey focused on assessing the frequency of mosquito nuisance incidents over the last decade, the identification of potential mosquito breeding habitats, and the implementation and effectiveness of control strategies. The findings revealed that 14 out of 257 LAs reported mosquito nuisance incidents in the past decade, 8 local authorities encountered complaints within the last year. Moreover, 70 Las identified potential mosquito breeding sites within their jurisdictions, and four reported conducting control measures in the past year. Many local authorities were unaware of accurate mosquito habitats, with some mistakenly identifying open bodies of water as suitable breeding sites. This limited awareness, both among LAs and the public, may result in underreporting of mosquito nuisances and misconceptions about their impacts. Less than 1% of LAs reported dedicated budgets for mosquito control, potentially reflecting the sporadic nature of mosquito nuisances in some areas. The reasons for non-responses and inconsistencies in data recording require further investigation, as they may impact data accuracy and hinder effective mosquito management. To address these challenges, there is a pressing need for increased education and awareness campaigns on mosquito habitats and issues. Improved recording procedures and dedicated budgets for mosquito control are essential to mitigate mosquito-related problems effectively. Furthermore, enhancing communication and collaboration between local authorities and external pest control organisations can significantly improve the management of mosquito-related issues. This survey underscores the need to enhance awareness, and financial allocations for mosquito control by LAs. By addressing these areas, local authorities can take proactive steps to reduce mosquito populations and alleviate the future impact of mosquito nuisances on their communities.

Modeling spatial risk of malaria through probability distribution of *Anopheles atroparvus* and imported cases

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Malaria remains the most important infectious disease around the world due to its high incidence and mortality. The large flows of infected cases from endemic into non-endemic malaria regions like Europe have resulted in a major public health concern due to sporadic re-emergence of autochthonous cases. This is possible because competent vectors of the genus *Anopheles* occur in countries where malaria is not endemic. To estimate the geographic variation in the risk of autochthonous transmission in Spain, we first, confirmed by molecular assays that *An. atroparvus* s.s., the historic malaria vector in the country, was by far the most common and widespread species within the *Anopheles maculipennis* complex in Spain. Then, we modeled the potential distribution of *An. atroparvus* across Spain using the ensemble of eight machine-learning techniques based on presence/absence data (5749 records between 1995 and 2020) and relevant environmental parameters. We subsequently combined this map with the number of imported malaria cases (5718 between 2005 and 2020) in each municipality to detect the geographic hot spots with a higher risk of local malaria transmission. The malaria vector occurred preferentially in irrigated lands characterised by warm climate conditions and moderate annual precipitation. Some areas surrounding irrigated lands in North-East Spain (e.g. Zaragoza, Logroño) as well as mainland areas (e.g. Madrid, Toledo) and the South (e.g. Almeria, Huelva) presented a significant likelihood of *An. atroparvus* occurrence, with a large overlap with the presence of imported cases of malaria. Most of the imported cases derived from travelers from African regions (Sub-Sahara zone) which imported infections by *Plasmodium falciparum* (83.0%), *P. ovale* (2.7%), *P. vivax* (3.7%) and *P. malariae* (1.7%). The overlap between incidence rates of imported malaria and the high probability of occurrence of malaria vectors allowed to identify risk areas for autochthonous malaria transmission, providing fundamental information for stake holders that will favour vector control and raise awareness to improve the detection and treatment of imported cases.

Abacus: a rapid and reliable method to estimate larval abundance of container mosquitoes in the field

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Stegomyia mosquitoes (*Aedes albopictus* and *Aedes aegypti*) are important vectors of arboviruses (dengue, chikungunya, Zika and yellow fever). These mosquitoes develop mainly in artificial containers, allowing them to proliferate in urban and peri-urban environments. Knowledge of the vector productivity by breeding sites is important for mosquito control operators (MCO). Traditional larval indices are the reference, but rapid and accurate methods are necessary to assess vector population abundance (in addition to presence) in artificial breeding sites. The abacus method, initially developed for marsh mosquitoes, was adapted to the container mosquito. The abacus method consists of a visual comparison between the abundance of larvae placed in a standardised container and reference images of abundance classes. Abundance classes range from 1 to 5 for the abacus 5 and from 1 to 10 for the abacus 10. The reliability and efficiency of abacus was evaluated on *Aedes albopictus* and others container mosquito species. The influence of several factors on the abundance estimations of immatures stages was tested: the level of experience of the reader, the number of species, the quantity of organic matter, the number of stages and the number of larvae. In total, more than 30 000 larvae and 1800 pupae were used to build up nearly 200 tanks and take more than 600 readings. All the variables studied influenced the readings, with a tendency to overestimate (number of species) or underestimate (quantity of organic matter, diversity of immature stages, experience of the reader). Extreme estimation errors are caused by mosquito species misidentification: simple identification criteria for live larvae have been selected to ensure that anyone can correctly identify the species in the field, in France and possibly in many other countries in temperate and tropical areas. The abacus method (identification and abundance estimation) required less time than the larvae-by-larvae counting method and is suitable for widespread application in the field by MCO or in participatory science systems.

Mosquito Alert: Schools collaborate in vector control through citizen science

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Mosquito Alert is a platform that enables users to report the presence of mosquitoes, their breeding sites, and whether they have been bitten through a mobile app. This online educational project aims to raise awareness among students about mosquito biology and mosquito-related disease issues and promote their engagement in citizen science. The project started in 2016 with a few schools in Spain and has experienced significant growth, expanding first at the national level and later, in 2020, to the Netherlands. The number of participants has steadily increased, with approximately 10,000 students participating in total. The project caters to different age groups, offering three versions: primary school, lower secondary, and upper secondary. Each school has its own access to a virtual classroom, where students encounter the various stages of the project. These stages are directed by teachers and are divided into three blocks: learning, data collection, and the creation of final products. As of the current date (2023), we have collaborated with nearly 200 educational institutions, which have reported over 3,500 breeding sites using the application and have produced more than 1,500 final products as part of the project. Furthermore, we have gathered valuable qualitative insights through surveys that highlight the project's appeal for various reasons, including its connection to real-world issues and the opportunity it provides for students to actively participate as citizens in scientific research. We have achieved promising results and maintained strong engagement from educational institutions and teachers, allowing us to continuously improve the project's learning outcomes. Our main challenge now is to find sustainable ways to expand the project, both in current and new locations. This not only empowers individuals with essential knowledge and skills but also contributes to the larger goal of enhancing public health and preventing diseases through informed communities.

Session 5. One health: challenges and perspectives

ORAL PRESENTATIONS

Results of entomological surveillance 2022 for West Nile and Usutu virus in northeastern Italy

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In Italy, the surveillance system for West Nile virus (WNV) and Usutu virus (USUV) combines human surveillance with veterinary and entomological surveillance. We report the results of entomological surveillance in 2022, a year characterised by high circulation of both viruses. Entomological surveillance was carried out all over the flatlands of northeastern Italy from May to October using 78 CDC-like traps baited with CO_2 and 8 Gravid Traps. The traps run for one night every two weeks. Pooled female specimens of *Culex* genus, *Aedes albopictus*, and *Ae. caspius* were tested for viruses by retrotranscription Real-Time PCR screening for the genus Flavivirus followed by sequencing. In 2022, WNV activity started very early (7 June). In total, 103 611 mosquitoes were captured (2568 pools analysed). The total number of mosquitoes captured was lower than in previous years (122 056 in 2021), but the number of WNV and USUV positive pools was higher than in 2021 (110 positive pools vs. 20 and 18 positive pools vs. 43 for WNV and USUV, respectively). Also different from previous years is the circulation of lineages 1 and 2 of WNV and USUV at the same sites and time; in 2021, this happened only in one capture site. Phylogenetic analyses on WNV 1 complete genome showed that the virus detected in 2022 was closely related to that identified in 2021 (99.9% sequence identity) forming a distinct cluster within the Mediterranean subtype of clade 1a. Epidemiological and phylogenetic data suggest that the new variant of WNV 1 was introduced in 2021 at a single site and then spread in 2022 throughout northeastern Italy. Lineage 2 clusters with the south-central European clade (identity ≥99.6%) circulating in Italy since 2016. USUV characterised in 2022 cluster within the EU2-A lineage, which includes all the most recent Italian sequences. The results of entomological surveillance in a year of high viral circulation showed that mosquitoes are probably the best sentinel to highlight the circulation of these viruses. Therefore, it is important to implement this type of activity in the One Health approach for Arboviruses surveillance. This work was funded by the Veneto and Friuli Venezia Giulia Regions.

Investigation of *Dirofilaria* spp infection in *Culex pipiens* mosquitoes and dogs across the Attica Region

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Dirofilariosis, a significant parasitic disease impacting dogs and cats globally, exhibits a notably elevated prevalence in the northern parts of Greece. Epidemiological models indicate the suitability of temperature in various Greek regions for disease transmission. Our objective was to evaluate the presence of the parasite in the Attica region, located in the eastern part of Central Greece, focusing on its circulation among dogs and mosquito vectors. We conducted a comprehensive study analysing 2090 blood samples from dogs using an ELISA-antibody test. In addition, 913 blood-fed *Culex pipiens* mosquitoes were examined as potential vectors. These mosquitoes were collected from 55 different areas in the Attica Region using BG-sentinel traps equipped with BG-Lure and CO_2 gas cylinders. Positive and/or suspected results were detected in nine distinct areas among the dog blood samples. Additionally, during the study period, a clinical case was reported, involving a dog fatality attributed to dirofilariosis. Out of the 224 pooled mosquito samples, 23 from 12 different areas were tested PCR positive for *Dirofilaria* spp. Based on the results, four areas within the Attica region exhibited a combination of positive and/or suspected *Dirofilaria* serological results in dog blood samples and positive blood-fed mosquitoes. Our preliminary findings demonstrate the emergence of dirofilariosis in the Attica region, both among dog populations and in mosquito vectors. These results emphasise the importance of continued monitoring and public awareness efforts to manage the spread of this parasitic disease. Given the zoonotic potential of dirofilariosis, understanding its epidemiology in different sites in Greece (a highly endemic country) becomes really important and should be addressed. To further pursue this goal, an One Health approach, integrating human, animal, and environmental health, should be adopted.

Integrated approaches for mosquitoes blood meal analysis, species identification and virus screening in Spain

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The correct species identification in mosquitoes in combination with analysis of their blood meals and pathogen screening is essential for disease control programmes. In this study, we employed an integrated approach to examine the mosquito diversity, their blood meals and pathogen detection in three different aquatic ecosystems in La Rioja, Spain. Mosquitoes were collected using BG Sentinel traps baited with CO2 and aspirators. Vector and host DNA was identified from RNA extractions using IonTorrent technology, and PanFlavi and PanAlpha PCRs were carried to detect flavivirus or alphavirus. The composition and the abundance of the species varied depending on the wetland being *Coquillettidia richiardii* (43.7%) the most abundant in La Grajera, *Aedes caspius* (47%) in Las Cañas wetland, and *Culex pipiens s.l.* (64.4%) (in the Iregua river). In total, we analysed 475 specimens with different states of blood engorgement. As a whole, we collected 20 species of mosquitoes, and the use of COI DNA barcoding contributed to the correct identification of morphologically challenging species such as taxa within the genera *Anopheles* and *Culex*. Fifteen species with bloodmeals in their abdomen were analysed, of which 37 hosts ranging from birds, mammals to amphibians were identified. *Culex pipiens s.l.* desmostrated to have a larger number of hosts (24 species), with birds being the most numerous (20 hosts); the common wood pigeon (*Columba palumbus*) was the most utilised. No viral RNA were detected from the PanFlavi and PanAlpha PCRs that we performed. In conclusion, we demonstrate the importance of using molecular techniques to support species-level identification and analysis of blood-fed mosquito females to maximise the information obtained in studies investigating host feeding patterns during vector surveillance.

Assessing the risk of mosquito-borne diseases in Scotland and their response to environmental change

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Climate and other environmental changes are driving the expansion of mosquito vector borne diseases (VBDs) into areas of Europe previously unsuitable for transmission; with many being derived from avian populations. This presents a growing risk of VBD establishment in the United Kingdom due to presence of competent vectors and zoonotic pathogens in resident and migratory birds. Ability to detect and respond to emergence is however constrained by major gaps in national surveillance; with current activities restricted almost entirely to England and Wales. In contrast, almost no data on mosquito and avian reservoirs are available for Scotland. Here we will give an overview of the 'Mosquito Scotland' project; a new multidisciplinary research programme designed to address this gap through comprehensive investigation of mosquito vectors and avian reservoir populations in Scotland. Our goal is to assess potential for VBD transmission under current and future environmental conditions through integration of entomological, pathogen and wildlife surveillance and modelling. Activities include surveillance of mosquitoes over 2 years in geographically and ecologically representative wetland habitats, and across a rural-urban gradient extending from Glasgow City. Mosquito and avian reservoirs will be screened for up to 40 VBD pathogens and endosymbionts using a novel micro-fluidic PCR approach. Additionally we will establish laboratory colonies of *Culex pipiens* from wild populations and use them to assess the competence of Scottish mosquito populations for West Nile and other viruses emerging from wildlife reservoirs in Europe (e.g. Usutu and Sindbis virus). In combination, these data will be used to develop distribution maps for mosquito vectors and model the likelihood of zoonotic VBD establishment in Scotland under current or future conditions. The presentation will focus on the design and implementation of this interdisciplinary One Health mosquito VBD surveillance programme, and experiences from the first season of data collection.

Culex pipiens biotype *pipiens* and *Cx. torrentium* show opportunistic host-feeding patterns

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In Europe, *Culex pipiens* and *Cx. torrentium* are known as vectors for arthropod-borne pathogens like West Nile virus (WNV), Usutu virus (USUV) and Sindbis virus (SINV). To comprehend transmission cycles, the understanding of mosquito host-feeding patterns is fundamental, enabling the infection of the mosquito and the subsequent transmission of the pathogen to other hosts. In literature, both species are frequently described as ornithophilic. We collected blood-engorged female mosquitoes in Germany using different traps, including BG Sentinel traps, BG Pro traps, EVS traps and aspiration from resting sites. Morphologically identified *Cx. pipiens* s.s./*Cx. torrentium* were typed to species-level using a DNA-typing assay. Blood meal hosts were identified using barcoding PCRs targeting the cytochrome *b* or 16S rRNA gene fragment with afterward sanger sequencing. Our results demonstrated, that *Cx. pipiens* s.s. and *Cx. torrentium* have a very broad host-range feeding on a high species diversity of mammals and birds. More than 50% of the detected host were mammals dominated by cattle, deer, wild boar and human. In summary, *Cx. pipiens pipiens* and *Cx. torrentium* must be opportunistic feeders with no specificity for birds. Therefore, the two species have to be considered both, potential enzootic and bridge vector for WNV, USUV and SINV.

Evaluation of methods using honey dipped FTA-Filter for detection of mosquito-borne viruses

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Arboviruses, transmitted to humans and animals through the bites of infected mosquitoes, are a major public health concern worldwide, as they can cause a wide range of diseases. Honey-baited FTA cards in mosquito traps have been used to detect the presence of arboviruses, but the sensitivity is still not clear. We aimed to develop a sensitive and cost-effective method for nucleic acid isolation with FTA cards to detect low amounts of viral RNA. Honey-dipped FTA cards inoculated with Sindbis virus were used to evaluate two extraction methods, TRIzol extraction and Qiagen´s RNeasy Mini Kit and analysed with RT-qPCR. There were only slight differences between the two extraction methods where TRIzol extraction resulted in detectable viral RNA when low concentrations of virus was used but the methods were comparable for higher concentrations. The stability of viral RNA on FTA cards was observed with Sindbis and Usutu viruses. Virus suspension was applied to honey dipped FTA cards and incubated in room temperature up to two weeks before extraction and RT-qPCR. No depletion of viral RNA was seen over this time. As a method to look for a broader range of viruses, family primers were used targeting Alphavirus, Orthobunya virus or Flavivirus genus. Sindbis, Usutu, Inkoo, and O'nyong'nyong viruses were used for the evaluation of family primers with PCR and Sanger sequencing. To field test the methods, honey dipped FTA cards were used in CDC light traps in eight locations in Kenya. RNA was extracted and family primers were used to screen the samples. The resulting amplicons were sequenced using Oxford nanopore minION long read sequencing. Sequences indicate that one of the samples contain an alphavirus, O'nyong'nyong virus. Sequences from an additional Orthobunya virus of the California serogroup, yet to be determined was also found in the samples. Our results indicate that FTA cards can be used to detect mosquito borne RNA viruses without losing RNA if stored at room temperature and can thus be implemented when a cold chain isn't practical. For virus detection, several methods can be used depending on the scientific question. Field trial in Kenya with samples delivered to Sweden by post without cold chain confirm that the method can detect circulating mosquito borne viruses.

Session 5. One health: challenges and perspectives

POSTERS

Spread of *Dirofilaria immitis* and *D. repens* in Europe

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Dirofilaria immitis and *D. repens* are mosquito-borne nematodes with dogs as primary reservoir and amplification hosts. However, infections in a range of other animals including cats and humans a regularly observed. While both pathogens circulate in southern Europe for centuries, in the last few decades a drastically spread towards central Europe is observed, threating animal and human health. In this study, we compiled published distribution data of *D. immitis* and *D. repens* infection cases in Europe and analysed their spread over the last 20 years. Additionally, we used temperature data and the mechanistic transmission model using the *Dirofilaria* development units to predict areas at risk of transmission. Most *D. immitis* infections were reported in dogs, while most *D. repens* cases were diagnosed in humans. Compared to the time before 2000, many more countries are currently affected by *Dirofilaria* spp. circulation. Similarly, while areas at risk for transmission were mostly identified in southern regions of Europe until 2000, currently many parts of central, eastern, and western Europe are at risk of autochthonous *Dirofilaria* transmission. These findings suggest that *D. immitis* and *D. repens* are a growing health concern for animals and humans in Europe and that the continuously climate warming will lead to a further spread and increased circulation.

The risk of dengue virus emergence in Romania, in the conditions of climate warming

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Abstract In order to be able to prevent future dengue epidemics in Romania, it is necessary to establish the bioclimatic conditions which can cause the occurrence of dengue outbreaks. In this sense, we studied the climate changes in several regions in Romania, in order to be able to establish the risk areas. In this study, we proposed the use of bioclimatic indices to estimate the favourable periods for the development of *Aedes albopictus* mosquito populations and the incubation periods of the virus inside the vector. In the present study we aimed to determine the population of *Aedes albopictus*, in terms of the risk of transmission of dengue virus, by calculating the number of days favourable for the formation of the mosquito population, in ideal conditions with temperatures of at least 27°C, which also coincides with a favourable period for the replication of the virus inside the mosquito, which can contaminate the host on which it feeds, causing the disease. The index is important in order to calculate the maximum possible number of eggs per year under ideal local temperature conditions. We introduced into the study the potential development period index of *Aedes albopictus* (MPI) as an indicator of potential mosquito development periods according to temperature conditions. It sums up all the monthly periods (MPIm) required for a mosquito larva to reach maturity. The annual hatching potential index is calculated as a sum of monthly hatching potential indices. The second index defined by us is the potential index of infestation with the dengue virus (PII), which calculates the maximum possible periods from the infection of female mosquitoes to the first day when they can transmit the virus. The maximum possible annual periods are 24 hours at a temperature above 27°C. The results estimate an increase in temperatures in Romania of up to 2.6°C in HS and up to 0.4°C in LS (low scenario), with an increase in the period of virus replication within the vector from June to October in HS (high scenario), and from May to September in LS. Also, in 2022, *Aedes albopictus* was reported in a new county, where it was not identified at the last monitoring in 2020. The species Aedes albopictus has been identified in the Big Island of Braila, on the arm of Măcini-Dunărea Veche, most of the females being caught with a hand aspirator, directly from the human host, in the 14–16°° range, showing an increased aggressiveness of the females. The rapid spread of this invasive species and the need to implement monitoring and control programmes for the *Aedes* population in Romania are emphasised.

Session 6. Surveillance and management of black flies, sand flies, ticks, and others

ORAL PRESENTATIONS

A survey of sand flies across their known northern distribution range limit in Western Europe, summer 2023

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Sand flies (SF) are vectors of *Leishmania* spp. protozoa and Phleboviruses. In this study, we sampled areas of Northern France, the Benelux and neighbouring Germany, encompassing the known SF distribution range northern limit. It this area, very limited and no recent data on SF occurrence exists. Sampling was performed in July and August 2023 using CDC-light attraction traps (with or without CO_2), 'sticky' interception traps, and human landing captures, in 179 sites in 48 localities (22 NUTS3 geographical subdivisions). A total of 55 sand flies were collected in 11% (20/179) of the sites sampled, including 16 sites in France, 2 in Luxembourg and 1 in Germany. This is the first time that SFs are reported in Luxembourg and in Trier-Saarburg county of Germany. No SFs were detected in Belgium and in the Netherlands. Species samples included 39 *Phlebotomus mascittii* and 16 *Ph. perniciosus*, the later found only in Savoie, the southernmost NUTS3 sampled. All specimens were captured with light traps except for 2 human landing catches. The proportion of SF-positive sites was not significantly associated with environmental variables (p>0.05) although it numerically decreased with increasing altitude, was highest in rural sites and in traps close to human and animal buildings. The study confirms and provides new evidence of SF occurrence in areas of Western Europe and highlights the need for awareness of potential autochthonous transmission of SF-borne pathogens in these areas. This survey was performed within the VectorNet project and the capacity building activities, funded by ECDC and EFSA, with trainees from Belgium (Schneider A., Vanslembrouck A, Van Bortel W.), France (Bersihand S., Binder C., Cornen T. Rey D., Rozier Y., Zambianchi D.), Luxembourg (Weigand A.) and the Netherlands (De Boer K., Ibañez-Justicia A., Stroo A.).

The Phlebotomine sand fly fauna of Switzerland revisited

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Sand flies (Diptera: Psychodidae, Phlebotominae) are widespread in Europe, being particularly common in the Mediterranean region but rare north of the Alps. Thus, Switzerland is an opportune place to investigate the sand fly fauna on both sides of the Alpine crest, in southern sub-Mediterranean and northern oceanic temperate climate. We re-investigated the Swiss sand fly fauna 20 years after the last studies, with the aims to assess changes in composition, altitudinal distribution, abundance and seasonality. Thirty-eight sites were investigated with light traps and/or interception sticky traps in four years, between 2009 and 2016, from early July to early September. Abundances were calculated as the number of sand flies collected divided by the sampling effort. Specimens were identified to species level by morphology, MALDI-TOF mass spectrometry or barcoding. Ninety and 380 specimens were caught by light traps and sticky traps, respectively, at 15 collecting sites. Four species were identified in southern Switzerland. *Phlebotomus mascittii*, *Ph. perniciosus* and *Sergentomyia minuta* were confirmed in the canton of Ticino, and *Ph. mascittii* for the first time in the neighbouring canton of Grisons. Also, *Ph. neglectus* is for the first time reported, though at a very low density compared to *Ph. perniciosus* at the same site in the very south of the country. Its presence in Ticino supports the northward spread observed in Italy. *Phlebotomus mascittii* was observed at the northernmost places and highest altitude; it occurred through the 250–750 m a.s.l. elevation range. Highest densities were observed within weeks 28–29 (mid of July) for all species except *Se. minuta* (weeks 34-35). The overall density of sand flies in Ticino was lower than in a previous study performed 20 years ago, and it is much lower than what can be found in Mediterranean countries. Sand flies (*Ph. mascittii*) were detected north of the Alps at one site only, endorsing a historical report. Overall, the low density of *Ph. perniciosus* and very low density of *Ph. neglectus* suggest that canine leishmaniosis may not be an important disease risk in Switzerland. Acknowledgment: We acknowledge the Swiss Federal Food Safety and Veterinary Office as sponsor of the National Centre for Vector Entomology.

Black flies (Simuliidae): best practices for a major emerging pest in southern Europe. Part 1: surveillance

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Black flies have become in the last decade one of the main emerging pests in Spain. Due to several factors (e.g. modification of hydrological basins and riverside vegetation, and increase in water quality), nuisances by mammophilic and anthropophilic species are increasing. In southern Europe, no black fly species has been confirmed as a vector for any known zoonotic disease. However, Simulids may cause a significant impact on public health due to the annoyance of its bites, which often lead to severe allergic reactions in susceptible populations. Biting black fly populations cause a threat to the economy, tourism, fruit picking as well as agronomic productions by generating morbidity on livestock. As such, proper pest control programmes are needed in affected areas to shield public and animal health. An appropriate black fly management should include surveillance of both pre-imaginal and adult populations. Even though black flies are a major pest concern in southern Europe, no major general guidelines have previously been designed for its surveillance, making results hard to compare between different management programmes. Randomised or selective surveillance is employed for the population density evaluation of pre-imaginal stages. Pre-imaginal stages gathered from submerged vegetation, stones or garbage are used to estimate population. However, there is currently no method available that accurately estimates the total number of black fly individuals present in a specific river basin. Surveillance should be conducted periodically, particularly during the peak activity periods of adults, which occur between April and October. Taxonomic identification is regarded as one of the foremost challenges in black fly surveillance programmes. Species identification is generally either not performed or restricted to pupae populations. However, trapping and identifying adult black flies is essential for determining the species responsible for biting both livestock and humans. The objective of this work is to identify the best practices and methods for black fly surveillance, which are necessary for the establishment of proper control interventions in rivers of southern Europe.

Black flies: best practices for a major emerging pest in southern Europe. Part 2: treatments and evaluation

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In general, control programmes against black flies are solely based on liquid applications of the larvicide *Bacillus thuringiensis* var. *israelensis* (*Bti*) on river basins. While several variables could be considered, the general dosage determination is conducted based on river flow rates and larval densities. Nevertheless, several cryptic variables could play a major role in determining the general efficiency of treatments such as turbidity of water, wind speed, etc. The effective range of effect and periodicity of treatments should also be considered before and after interventions and must be coordinated with hydrological stakeholders to improve the efficiency of any intervention. Success of the operation is evaluated by pre- and post- treatment larval counts. In highly affected areas, treatments may be needed every 7 to 15 days to properly maintain low larvae population densities. It is also advisable to implement preventive treatments during winter or early spring to reduce the initial surge in adult population. The monitoring of medical care for insect bites can serve as a general evaluation of the control programme. Alternatives to *Bti* application by foot or boat are contemplated, such as aerial treatments. The real cost-efficiency of aerial application needs to be properly evaluated. Furthermore, the mechanical removal of vegetation masses in direct contact with the waterline could serve as a strategy when combined with *Bti* treatment, enhancing the control efficiency. Occasionally, adulticide treatments are employed to reduce population levels in heavily affected environments or risk areas. Nevertheless, those interventions are not recommended, due to its brief effectiveness and the great effect on non-target species. In addition to the standard larval control methods, there is also a need to explore novel control alternative strategies such as adult mass trapping. Effective coordination and collaboration with local stakeholders are crucial in devising strategies to enhance the efficiency of interventions. In Spain, the control of black fly populations is carried out at the municipal level, but this pest needs to be coordinated also at a regional level, related to the hydrological system, even across political borders.

Stable fly management at a donkey sanctuary in Spain: a new IPM approach

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The stable fly, *Stomoxys calcitrans* (Linnaeus), is a significant livestock pest globally. Its larvae develop in various substrates such as rotten plant material or manure. The emerged adults bite both animals and humans to feed on blood. Costs generated by these flies within the livestock sector has been estimated at US$2.2 billion/year in United States. In France the losses in the meat sector are estimated at €145 million per year, with an additional average annual loss of €234 million in the dairy sector. These losses are due to the nuisance caused by painful bites, which prevent animals from feeding properly, and have the potential risk of transmission of pathogens. Blood loss can be very significant during peaks of fly abundance. While chemical control by using insecticides is often used, this method harms the environment and faces resistance problems. Flies have demonstrated resistance to insecticides both phenotypically and genetically. We propose an integrated pest management (IPM) solution for stable fly control. Our approach involves using a combination of natural enemies to control the immature stages, along with a specific trapping system of adults. The Stomoxycc® trap is used for mass trapping of adult *Stomoxys*. As natural enemies of stable flies, we use the predatory mite *Macrocheles robustulus* (Berlese), which feeds on the eggs and first larval stages of flies. Additionally, we utilise two species of parasitoids, *Spalangia cameroni* Perkins and *Muscidifurax raptor* Girault & Sanders, which lay their eggs inside the fly pupae. Both predators and parasitoids are released bi-monthly on animal bedding. This IPM approach has been implemented in a large donkeys/mules/horses sanctuary in the south of Spain (El Refugio del Burrito, Bodonal de la Sierra, Extremadura) in June 2022. Results will be discussed during this conference.

Integrated approaches for surveillance of ticks and tick-borne diseases of livestock importance

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Ticks are of medical and veterinary concern due to their role as vectors of a wide range of pathogens, including viruses, protozoa and bacteria. Therefore, accurate identification of tick species is essential for surveillance and disease control programmes. In this study, an integrated approach was implemented for the monitoring of ticks and tick-borne diseases of livestock and wildlife. Morphological traits in combination with genetic variability within the cytochrome *c* oxidase I (*CO*I) gene were employed to develop a DNA barcoding library for the identification of tick species in the United Kingdom. This also included relevant exotic taxa. In addition, molecular analyses were carried to detect specific pathogens either in ticks, or tissues and/or blood samples taken from infected animals. In total, 30 tick species were analised for the developing of the *COI* DNA barcoding gene profile in the genera *Amblyomma*, *Argas*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Ornithodoros* and *Rhipicephalus*. Phylogenetic analysis confirmed discrete clustering of sequences according to species, except for *Rhipicephalus sanguineus s.l.*, *Ixodes vespertilionis* and *Ixodes nr. affinis*. Examination of fox carcasses (*n*=13) showed they were carrying *I. canisuga*, *I. hexagonus* and *I. ricinus*, of which nine carcases (69.2%) were infected with *Babesia vulpes*. Testing of blood and tissue taken from cattle with febrile disease (*n*=22) detected *Babesia divergens* in four samples (18%) and *Anaplasma phagocytophilum* was detected in a sheep sample. Further specimens of *I. ricinus* from National Parks and Areas of Outstanding beauty in Wales and England revealed the presence, albeit with low prevalence, of *B. capreoli*, *B. divergens*, B. *venatorum* and *B. odocoilei*. Testing of RNA samples using a PanFlavi or TaqMan Real Time qPCR revealed one sheep infected with Louping Ill virus; the same sample was infected with *A. phagocytophilum* after testing using an specific PCR. In conclusion, all results showed that the combination of morphology, *COI* DNA barcoding, and targeted molecular assays was an effective approach for distinguishing ticks, revealing cryptic diversity, and the detection of tick-borne pathogens in ticks and animal species.

'Paparra Balear' a citizen science project for the surveillance of ticks in the Balearic Islands (Spain)

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Vector species of ticks and the pathogens of human and veterinary importance that they transmit have been poorly investigated in the Balearic Islands. One of the limitation for the surveillance of ticks is time constraints. There are few methods based on traps and therefore, most of the sampling is conducted using time consuming methods, such as vegetation flagging or direct collection from hosts. The citizen project named 'Paparra Balear' aims to collect tick specimens by citizens in order to expand the knowledge about tick species and their distribution in the Balearic Islands (Spain). Ticks are Arthropoda which are easy to recognise by the general public, collection is feasible and safe by non-specialised persons and they are easy to be preserved. The project also aimed to raise awareness about tick bites, the potential pathogens they transmit and the correct procedure for tick removal from humans and pets. The project was launched in July 2023 via a mass media (TV, radio and newspapers) and social network campaign (Twitter/X). In order to increase the visibility of the project, informative posters (up to 500) were also distributed to most pharmacies of the islands, thanks to the College of Pharmacists of the Balearic Islands. Veterinary clinics were also included in the project via the College of Veterinaries of the Balearic Islands. In all cases, information about the correct way to remove and preserve tick specimens was provided. The contact methods provided for the collaborators were via telephone and email. The project is still ongoing and up to early September 2023, 42 citizens have contributed by collecting ticks from vegetation, pets and humans. About the methods for contacting, 19 collaborators contacted via email, while 22 contacted via phone. The tick specimens (up to 253) were collected from Mallorca (*n*=239) and Menorca (*n*=14). Specimens were obtained from 19 municipalities of Mallorca and four from Menorca. No specimens were received from Ibiza. The three top municipalities in number of collected ticks were Muro, Llucmajor and Palma, all located in Mallorca. Most of the ticks specimens were collected in rural areas. The most abundant tick species collected was *Hyalomma lusitanicum*, followed by *Rhipicephalus sanguineus* s.l. and *Hyalomma marginatum*. All specimens were preserved for future analysis of the pathogen load. We discuss about the pros and cons of citizen science projects for collecting ticks.

Risk evaluation of mosquito species and their effects on human and animal health in Qatar

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Mosquitoes are recognised as significant vectors posing a threat to both human and animal health, as they facilitate the transmission of life-threatening pathogens, such as malaria plasmodia and arboviruses. In light of Qatar's rapid population growth, ongoing urbanisation, and technological advancements, the government is actively pursuing measures to safeguard human and animal well-being through early detection and effective vector management. This research endeavor aims to elucidate the identity of mosquito vector species, delineate their geographic distribution across various municipalities in Qatar, and undertake a comprehensive risk assessment to comprehend the dynamics of disease transmission. A total of 12 000 specimens were collected utilising CDC traps (CO_2-baited) and Blackhole traps (applying UV-light) during the period spanning from January 2022 to January 2023. These collections were made from diverse sample locations, encompassing animal farms, agricultural farms, and gardens situated within all the eight municipalities in the state of Qatar. The traps were typically deployed and activated prior to sunset and deactivated after sunrise. Subsequently, the collected samples underwent morphological identification, facilitated by taxonomical keys. Genomic DNA was extracted, followed by specific PCR targeting the mitochondrial COI sequence. The PCR products were purified and subjected to DNA sequencing. The resultant COI sequences were submitted to the NCBI GenBank for species identification. Geographic Information Systems (GIS) were employed to cartographically represent the distribution of vector species within Qatar. To determine the posterior probability of various species, data were subjected to calculation using the Naive Bayes model. Through both morphological examination and molecular identification, it was ascertained that the following species were present: *Culex pipiens*, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *Cx. sitiens*, *Cx. theileri*, *Anopheles stephensi*, *Aedes caspius* and *Culiseta longiareolat*a. Many of these species may assume pivotal roles in exacerbating the risk of transmitting a variety of diseases within the region. The insights gleaned from these surveys bear immense significance in the formulation of a comprehensive local and national risk assessment plan and the establishment of a database aimed at enhancing vector management and surveillance.

Session 6. Surveillance and management of black flies, sand flies, ticks, and others

POSTERS

DNA barcoding Latvian mosquitoes (Diptera: Culicidae) reveals hidden species diversity

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Recently, some Central European countries reported the emergence of West Nile virus, transmitted by *Culex pipiens s.s.*/*Cx. torrentium*, that confirms a tendency of virus spreading from south to the north of Europe. A zoonotic parasite *Dirofilaria repens* became established and endemic during the last decade in the Baltic countries, including Latvia. An updated information on regional mosquito species composition and distribution is important to estimate the possible public and animal health threats. Information on Latvian mosquitoes is the most limited among the Baltic countries. The earliest studies of mosquito fauna in the present-day Latvia refer to the first half of the 19th century and the last records were reported in the 1970s. Later these data have been summarised, listing 25 species. Meanwhile, 37 mosquito species were recorded in Lithuania and 34 species in Estonia. The aim of this study was to confirm the historical species records and update the checklist of Latvian mosquitoes for the first time since 1979. Thus, 643 mosquito specimens (548 females, 40 males, 55 larvae) were collected in Latvia in 2017 using CDC traps baited with CO_2, human landing, manual aspiration and dipping techniques. All specimens were preliminary identified by morphology. In addition, 437 *Aedes* (*n*=434) and *Anopheles* (*n*=3) mosquitoes were subjected to the DNA barcoding using mitochondrial COI gene. Overall, 36 specimens from Maculipennis complex were identified to the species level by sequencing the ITS2 rDNA. A multiplex real-time PCR was used to identify 65 specimens belonging to *Cx. pipiens* s.s./*Cx. torrentium*. The presence of the following species was confirmed by DNA sequencing: *Ae. annulipes*, *Ae. cantans*, *Ae. cataphylla/Ae. leucomelas*, *Ae. cinereus*, *Ae. communis*, *Ae. cyprius*, *Ae. diantaeus*, *Ae. excrucians*, *Ae. flavescens*, *Ae. intrudens*, *Ae. punctor*, *Ae. sticticus*, *An. claviger* s.s., *An. maculipennis* s.s., *An. messeae*, *Cx. territans* and *Cx. torrentium*. The presence of *Ae. hexodontus* and *An. daciae* in the country was confirmed by DNA sequencing for the first time. Among *Culex* species, *Cx. pipiens* biotype pipiens is the dominant species in Latvia. Besides, *Cx. pipiens* biotype molestus and hybrids of the two pipiens biotypes were identified for the first time in Latvia. The presence of *Culiseta alaskaensis* was confirmed using morphological characters of hypopygium. *Aedes pullatus* was confirmed by morphology of adult female that was deposited in ZIN, St Petersburg. In total, 22 mosquito species were identified in the present study including two new species records.

Contribution to the knowledge of black flies (Diptera: Simuliidae) in the Republic of Moldova

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Most of black fly species feed on vertebrate blood and some of them are the confirmed vectors of viruses, bacteria, parasitic protozoans, and nematodes of humans and animals. In Europe Simuliidae are involved in the transmission of avian leucocytozoonosis, trypanosomiasis, filariasis, and human and bovine onchocerciasis. Furthermore, high biting activity of Simuliidae may cause acute irritation and toxemia among the poultry, cattle and humans. The historical records of black flies are absent in the Republic of Moldova. The closest well known black fly fauna of Romania consists of 71 species, belonging to three genera (*Prosimulium*, *Metacnephia* and *Simulium*) and the fauna of United Kingdomraine includes 93 species from five genera (*Prosimulium*, *Twinnia*, *Cnephia*, *Simulium* and *Stegopterna*). The purpose of this study was to update the data on the Simuliidae species diversity and ecology in the country. The field work was conducted between 2018 and 2023 in 19 regions of the Republic of Moldova. Various physico-chemical parameters of breeding sites were obtained and analysed. The black fly specimens were identified by morphology of larvae, pupae and adults. In total, 33 black fly species, belonging to the genus *Simulium* and 6 subgenera, were identified as follows: 1) *Boophthora*: *S. erythrocephalum* (De Geer, 1776); 2) *Byssodon*: *S. maculatum* (Meigen, 1804); 3) *Eusimulium*: *S. angustipes* Edwards, 1915, *S. aureum* Fries, 1824, *S. krymense* (Rubtsov, 1956), *S. maritimum* (Rubtsov, 1956), *S. rubzovianum* (Sherban, 1961); 4) *Nevermannia*: *S. angustitarse* (Lundström, 1911), *S. brevidens* (Rubtsov, 1956), *S. carthusiense* Grenier & Dorier, 1959, *S. costatum* Friederichs, 1920, *S. delizhanense* (Rubtsov, 1955), *S. fontium* (Rubtsov, 1955), *S. lundstromi* (Enderlein, 1921), *S. vernum* Macquart, 1826, *S. volhynicum* (Usova & SUnited Kingdomhomlin, 1990), *S. zakhariense* (Rubtsov, 1955); 5) *Simulium*: *S. baracorne* Smart, 1944, *S. bezzii* (Corti, 1914), *S. deserticola* Rubtsov, 1940, *S. dolini* Usova & SUnited Kingdomhomlin, 1989, *S. frigidum* Rubtsov, 1940, *S. intermedium* Roubaud, 1906, *S. kiritshenkoi* Rubtsov, 1940, *S. noelleri* Friederichs, 1920, *S. ornatum* Meigen, 1818, *S. palustre* Rubtsov, 1956, *S. truncatum* (Lundström, 1911); 6) *Wilhelmia*: *S. balcanicum* (Enderlein, 1924), *S. dahestanicum* (Rubtsov, 1962), *S. equinum* (Linnaeus, 1758), *S. lineatum* (Meigen, 1804), *S. pseudequinum* Séguy, 1921. Most probalby a number of species remain undiscovered due to insufficient survey of the major rivers Dniester, Prut and Reut, along with unexplored streams and small rivers. Further surveys are needed to clarify the black fly species diversity in the Republic of Moldova.

Tick distribution in the United Kingdom and possible expansion of *Ixodes ricinus* identified using passive surveillance

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Tick-borne disease risk is linked to the distribution of tick vector species. Thus, to assess disease risk and possible emergence, understanding tick distribution, seasonality and host associations is needed and this can be achieved through passive surveillance. The aim of this study was to use data from the United Kingdom Tick Surveillance Scheme to assess changes in tick distribution and understand which species of ticks are present in the United Kingdom and their distribution. Data was collected through passive surveillance between 2013 and 2020. Members of the public, GPs, veterinary practices and wildlife charities were able to submit ticks found on humans and animals along with information about location, date the ticks were found and the hosts they were found on. We also investigated potential changes in the distribution of *I. ricinus*, the main vector of several pathogens including *Borrelia burgdorferi* s.l. (agent of Lyme borreliosis) and tick-borne encephalitis virus. We selected records of *I. ricinus* bites on humans, dogs and cats in the United Kingdom and we divided the United Kingdom into 20 km × 20 km grids. We then investigated changes in the proportion of grids reporting a tick bite for each region using statistical models. Between 2012 and 2020, 7662 records were received and 37 tick species were detected. Most records were acquired in the United Kingdom with only 237 that were associated with recent overseas travel. The dominant species was *I. ricinus* and records peaked during May and June, highlighting a key risk period for tick bites. Other key United Kingdom species were detected, including *Dermacentor reticulatus* and *Haemaphysalis punctata* as well as several rarer species that may present novel tick-borne disease risk to humans and other animals. Imported ticks were also detected, including Crimea Congo Haemorrhagic fever virus vector *Hyalomma*. When investigating potential expansion of *I. ricinus*, 9.2% (range 1.2–30%) of grids had at least one record every year since 2013. Most regions reported a yearly increase in the percentage of grids reporting *I. ricinus* since 2013 and the highest changes occurred in the South and East England with 5–6.7% of new grids reporting *I. ricinus* bites each year in areas that never reported ticks before. Spatiotemporal analyses suggested that, while all regions recorded *I. ricinus* in new areas every year, there was a yearly decline in the percentage of new areas covered, except for Scotland. To conclude, these data allowed us to better understand the tick species present in the United Kingdom and their distribution. In addition, we identified a possible expansion of *I. ricinus* between 2013 and 2020.

Prevalence of *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum* and *Babesia* spp. in questing Ixodes ricinus in the United Kingdom

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Understanding the variation in pathogen infections rates in *Ixodes ricinus* ticks is important for assessing potential transmission of tick-borne diseases. The aim of this study was to conduct a snapshot survey at recreational areas in England and Wales to understand infection prevalence for three tick borne transmitted pathogens. Ticks were collected (with density of nymphs recorded for 10×10 m) each spring at 20 recreational areas between 2014 and 2019. Questing (*n*=4103) nymphs were individually tested for presence of three pathogens using PCR; *Borrelia burgdorferi* s.l. *Anaplasma phagocytophilum* and *Babesia* spp. Site-specific *B. burgdorferi* s.l. infection rates in *I. ricinus* nymphs varied from 0 to 9.7%, depending on locations, with an average infection rate of 4.0%. Genospecies composition of sequenced samples was 62.5% *B. garinii*, 20.3% *B. valaisiana* and 17.2% *B. afzelii*. *Anaplasma phagocytophilum* was detected in 3.6% of questing nymphs, ranging from 0% to 20% depending on the location. Ecotype I accounted for 87% of positive samples and ecotype II for 13%. *Babesia* spp. was detected in 0.38% of ticks. Ranging from 0% to 2.0%. Northern England and Wales had significantly higher infection rates for *A. phagocytophilum* (4.7% and 12.1%) whilst *B. burgdorferi* s.l. infection rates were higher in southern (5.4%) and northern (2.3%) England. Of 4,103 nymphs tested, 15 were found to be infected by *Babesia* spp., giving an overall prevalence of 0.38%. Four *Babesia* species were identified; *B. venatorum* (*n*=9), *B. capreoli* (*n*=4), *B. divergens* (*n*=1) and *B. odocoilei* (*n*=1). Results were presented to study collaborators in a series of workshops to provide an opportunity for knowledge exchange and feedback on the study.Working with a network of stakeholders can generate robust information on pathogen infection rates in ticks and provides a channel for communication on tick-related issues. Infection rates for the three studied pathogens varied depending on geographical locations and this study increases the knowledge on the prevalence of tick-borne pathogens in recreational areas across England and Wales. Future improvements to the study will include incorporating additional pathogens and the density of ticks to generate information on tick hazard.

Pathogens detected in ticks collected from foxes (*Vulpes vulpes*) in the United Kingdom

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Foxes are often found in urban areas in the United Kingdom, so the potential for the transmission of diseases to domestic animals and humans should be considered. More specifically, foxes can carry diseases transmitted by ticks, which are a threat as vectors of many pathogens including viruses, bacteria, and protozoa. Hence, it is important to assess the prevalence of tick-borne diseases (TBD) to understand their potential transmission and support disease control. In this study, ticks removed from fox carcases found across England, Wales and Scotland were tested for several tick-borne pathogens. Ticks were speciated morphologically prior to washing and homogenisation. DNA and RNA were extracted and assessed using molecular assays for tick-borne pathogens. Tick species were also confirmed using a COI gene PCR and subsequent sequencing. In total, 227 ticks were collected from 93 dead foxes between 2018 and 2022 (with no sampling between July 2019 and November 2021). Metadata collected included the map reference where the foxes were found. Pooling (n=2) was undertaken for nymphs from the same tick species and fox host. In total, 203 homogenates were tested (24 pools, and 179 adults). *Ixodes hexagonus* was the most abundant tick species found (72.7%), of which the majority (58.8%) were nymphs. *Ixodes ricinus* and *Ixodes canisuga* were detected less often (11.5 and 15.4%, respectively), and the majority were adult females (73.1 and 91.4%, respectively). One tick was in the larval stage, and only identifiable as *Ixodes* spp. A qRT-PCR for louping ill virus, which is tick-transmitted and endemic in the United Kingdom, was performed with no detection in any of the ticks tested. However, 8/203 tick DNA samples (seven individual ticks and one pool of two ticks) were positive for *Babesia* (2.61% [0-4.89]; CI, 95%); seven in *Ix. hexagonus* and one in *Ix. canisuga*. Sequence analysis confirmed that the majority of *Babesia* detections in *Ix. hexagonus* were *Babesia vulpes*, with only one detection of *Babesia microti*. The single detection in *Ix. canisuga* corresponded to 'Babesia badger type A'. Screening for *Anaplasma phagocytophilum* by amplification of the *msp*2 gene was also performed, with confirmation by sequencing pending. The results of this study provide further data on the prevalence of tick-borne pathogens in ticks found on United Kingdom foxes.

Sand fly entomological survey in outskirt areas with high incidence of human leishmaniosis in southern Spain

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Leishmaniosis, a globally distributed vector-borne zoonosis transmitted by sand flies, has significant implications for public and animal health. In Spain, among the 14 sand fly species identified, only five (*Phlebotomus ariasi*, *Ph. langeroni*, *Ph. mascittii*, *Ph. perfiliewi* and *Ph. perniciosus*) are confirmed or suspected vectors of *Leishmania infantum*. Nonetheless, few studies investigate these sand fly species in urban and peri-urban areas where a high incidence of human leishmaniosis has been reported, which is crucial for identifying potential reservoirs of *L. infantum*. This study aimed to evaluate sand fly presence and abundance, along with their blood-feeding preferences in Castro del Río (Córdoba, southern Spain), a rural municipality with a high incidence of human leishmaniosis. We conducted nine three-day sampling sessions, every three-week interval from May to October 2022. During these sessions, we used eight CDC-type traps baited with CO_2, strategically placed in eight locations within the municipality, such as farms, public gardens and residential areas. Sampling occurred from 8:00 p.m. to 8:00 a.m. Collected sand flies were gendered and species identified. Female sand flies were categorised into five levels (1–5) based on blood quantity and freshness to determine their blood-feeding preferences. In total, 1357 sand flies were captured (667 females and 690 males). Among the 1352 identified at the species level, four belonged to the *Phlebotomus* genus, with *Ph. perniciosus* being the most dominant (57.4%, *n*=776), followed by *Ph. papatasi* (1.9%, *n*=26), *Ph. ariasi* (1.6%, *n*=22) and *Ph. sergenti* (1.6%, N=21). The *Sergentomyia* genus was represented solely by *Se. minuta* (37.4%, *n*=507).Sand flies were captured in all traps at an average rate of 7/trap and 50/sampling. *Ph. perniciosus*, the primary *L. infantum* vector in Spain, was consistently captured in all traps. Interestingly, three located at peri-urban areas (indoor orchard, kindergarten playground and outdoor sports facilities) accounted for 64.2% (498/776) of total *Ph. perniciosus* captures. The dense and widespread distribution of *Ph. perniciosus* in these anthropogenic environments poses a significant risk to residents and regional mammalian species.

Understanding the vector role and distribution of Stomoxyini flies (Diptera: Muscidae) in the Balearic Islands

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Adult Brachycera fly bites can directly impact livestock by causing reductions in food intake, disturbances, painful bites, and blood loss. Indirectly, these bites can contribute to significant economic losses and production damage through pathogen transmission. The primary objective of this study was to assess the presence of blood-sucking flies and detect various pathogen-derived nucleic acids (piroplasm protozoans, Anaplasmataceae bacteria and Equine infectious anemia virus (EIAV)) in both oral and body parts of these flies. This was achieved using different PCR targets, namely the 18S rRNA, 16S rRNA, *groESL*, and *tat* genes, in six distinct farm environments located on the island of Mallorca in the Balearic Islands, Spain. In addition, distribution maps were created in Spain for the identified species, utilising not only our new records but also data extracted from a thorough review of scientific literature and digital databases such as iNaturalist, BiodiversidadVirtual, Biota or Gbif. Our study successfully confirmed morphological and molecular identification of two biting Stomoxyini species. The stable fly *Stomoxys calcitrans* was found to be a common pest species across all sampled environments, and the second species, the horn fly *Haematobia irritans*, was less abundant and had a more limited distribution. The selected PCR targets did not detect EIAV, protozoan, or bacterial pathogens in either fly species. However, *Wolbachia pipientis*, targeted using *wsp* genes, was identified in all pools of *H. irritans* (*n*=13) collected from the two investigated livestock farms. This study represents the first attempt to test for DNA and RNA pathogens in Stomoxyini biting flies in Spain. The discovery of the endosymbiotic *Wolbachia* organism in *H. irritans* represents the record from Europe and holds its significance for future research into the applications of this bacterium in biocontrol programmes. The distribution maps indicate the widespread presence of *S. calcitrans* in most of the Spanish territory, with numerous reports from digital databases underscoring the importance of citizen collaboration as complementary to traditional entomological surveillance. Limited records for *H. irritans* are available, indicating that these dipteran groups should be studied more extensively.

Session 7. Mosquito control in the Mediterranean region and wetland conservation

ORAL PRESENTATIONS

Drivers of the abundance of a local population of *Aedes albopictus* in Catalonia (Spain)

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The global dynamics associated with human mobility, urbanisation, international travel, and climate change have led to novel dispersal patterns of disease vector-carrying organisms, including *Aedes albopictus*. Originating in Southeast Asia, this vector has successfully colonised five continents in recent decades, with passive dispersal by vehicles contributing significantly to its rapid expansion. Furthermore, its adaptability to diverse environments has facilitated establishment in temperate regions. This work explores the impact of various climatic variables (e.g. temperature, rainfall, humidity) and the biological control agent *Bacillus thuringiensis israelensis* (*Bti*) during the year 2021 on the abundance of *Ae. albopictus* within the Jardí Botánic Marimurtra (Catalonia, Spain). The abundance of *Ae. albopictus* was found to be positively influenced by the cumulative temperatures and rainfall experienced during the three weeks leading up to the sampling week. Moreover, the presence of water in drains located within 150 meters of the BG-Sentinel traps had a positive effect on the vector's abundance. Conversely, the application of larvicides resulted in a temporary decrease in abundance, but this negative impact was mitigated or even reversed following rainfall peaks, which were hypothesised to cause a dilution effect. The study demonstrates that temperature and precipitation significantly influence the abundance of *Ae. albopictus*. Furthermore, precipitation can affect the efficacy of *Bti* treatments through a dilution effect. Based on these findings, optimising larvicidal control measures is crucial for reducing *Ae. albopictus* populations in the Botanical Garden. Treatment schedules should not solely rely on the manufacturers' recommendations but should be adjusted considering climatic variations and periods of intense precipitation. Implementing such adaptive strategies will be instrumental in curbing the expansion of this disease vector in affected regions.

Larvicide treatment optimisation at a botanical garden

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Aedes albopictus is a well-known vector of multiple diseases such as dengue, Zika, or chikungunya. This, together with the fact that it is an invasive species in Spain, makes the development and improvement of control techniques of paramount importance. In this work we develop a metapopulations ODE model for *Aedes albopictus* that incorporates Larvae, Adults and the concentration of a control agent (such as *Bacillus thuringiensis*) present in the water at each breeding site. In this mathematical framework, control interventions are modelled by Dirac's deltas, since we assume the interventions to be instantaneous compared to the time window studied. The model accounts for the thermal responses of the mosquito biological parameters and rainfall, which are known to shape mosquito population dynamics, as well as the spatial information concerning the distance between the different breeding sites. We use stochastic optimisation techniques to determine the optimal times for implementing treatments with respect to the seasonal climatic patterns. Additionally, in cases where the treatment of all breeding sites is not feasible due to budgetary or operational constraints, we identify traits in the spatial distribution or in the intrinsic characteristics of breeding sites that make them a priority for treatment. The impact of the control measures is assessed by estimating the amount of mosquitoes captured at BG-traps with and without control measures. The goal of our work is to obtain general guidelines for improving mosquito control using *B. thuringiensis* within the Marimurtra botanical garden (Blanes, Girona). These guidelines are aimed to be implemented during the upcoming 2024 mosquito season, but its applicability may be appropriate for other scenarios. The study is based on real data from 37 breeding sites, which are currently undergoing control measures at the Marimurtra botanical garden. By identifying these spatio-temporal cues of the breeding sites in a mathematical framework we expect to be able to improve the efficacy of current treatments while reducing the number of interventions needed to achieve them.

Cost-benefit analysis of *Aedes albopictus* population reduction methods evaluated in southern France

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The ongoing spread of *Aedes albopictus* in France results in a growing public health issue, with more locally-acquired cases of dengue and other *Aedes*-borne viruses regularly detected, mostly in southern France but also further north following colonised areas. Local and regional administrations rely mostly on larval source management communication campaigns, in an effort to encourage inhabitants of residential areas to help reduce population densities, as most of the breeding sites are found on private properties. However, such endeavour presents a number of challenges, starting with the difficulty of reaching all the productive water collections that are multiple and sometimes hard to find, and the resulting efficacy on mosquito density is unfortunately low in many instances. In the search for potential alternative or complementary methods against *Aedes albopictus*, the EID-Méditerranée along with research partners have been conducting several field evaluations in recent years around the city of Montpellier, including mass trapping, larvicide autodissemination and larval source reduction. We compared the benefits of the different prevention and control methods evaluated in terms of efficacy (i.e. mosquito abundance reduction), then put in perspective with their feasibility, acceptability, sustainability and implementation cost. The findings from the cost-benefit analysis are discussed, highlighting the most relevant and promising approaches for the reduction of *Aedes albopictus* populations in Southern France using currently available methods, and suggesting future field research directions to build evidence that could allow to refine integrated mosquito management strategies for France and other European and Mediterranean countries.

Achieving operational effectiveness on a regional scale: challenges and solutions of Western Greece's project

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Western Greece's Regional mosquito control project is pivotal in obtaining and maintaining a challenging balance between a) the mitigation of the threat of mosquito-borne diseases (with established annual WNV circulation and sporadic malaria occurrences) and b) the demand for mosquito absence due to the post-COVID ameliorated touristic product. However Western Greece, with its capital city of Patra (the third largest Greek city), is an area with increased vector populations of Culex and Anopheles genera and high epidemiological risk, as it combines vast wetlands (with more than 44 000 ha, Western Greece ranks first among all Greek regions), complex rural ecosystems that include rice paddies and intensive greenhouse crops and foci with high concentration of land workers from malaria-endemic countries. On the other hand, its ports and airports constitute the gateway to and from the Adriatic Sea and Western Europe, and with its extensive coastline and the infamous Archaeological Site of Olympia, it attracts tourists from all over the world; thus, elevating the demand for lower populations of nuisance mosquitos of the Aedes genus. To fulfil the mosquito control project's objectives, we utilise a wide range of tools and control methods over eight months each year, which include: 1. Reliable mapping and continuous evaluation of more than 30 000 breeding sites of 500 cities and settlements; 2. Iterations of larvae sampling (approximately 80 000 samples) and timely larvicide applications in the periurban environment and wetlands (from the ground and aerial with the use of drones and helicopters); 3. Repeated applications of larvicides in the stormwater basins of the cities; 4. Extensive door-to-door larvicide application in 45 selected high-risk settlements; 5. Extensive use of adult traps with 500 samplings annually, for the evaluation of adult mosquito abundance and its fluctuation over time 6. Functional sampling networks for the evaluation of epidemiological risk of WNV (WNV prevalence in sentinels – chickens, wild birds, horses, and adult mosquitoes) 7. Quality control methods. Data collected throughout the project's duration by field technicians and the project's scientific team, enhance an already reliable database with time-series data on the abundance of larvae and adult mosquitoes. By processing these data, we gain knowledge and a deeper understanding of the project's needs and demands. All and all, this presentation seeks to provide a clear view of our efforts toward mosquito control effectiveness and efficiency, along with a summary of mosquito control operations, their extent and intensity, the reasoning behind the timing of interventions, and the decision-making process for resource allocation.

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POSTERS

Results from a 3-years field evaluation of a mass trapping strategy against *Aedes albopictus* and *Aedes aegypti*

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Within mosquito species of public health importance, *Aedes aegypti* and *Ae. albopictus* are among the most important due to their extensive areas of presence throughout the world and their ability to transmit major human arboviruses, as well as being a major source of nuisance. Apart from using insecticides against these vectors (and its disadvantages: resistance phenomenon, hard to reach breeding sites), the only method used to reduce their abundance is communication and social mobilisation. Research into the use of inexpensive and simple traps, such as passive traps targeting gravid females, is needed to evaluate their efficacy and potential as complementary vector control methods. The VECTRAP project aimed to demonstrate the effectiveness, feasibility and sustainability of mass trapping against both species in 3 geographical areas (Mediterranean France, Alpine France, and the tropical island of Martinique) by using gravid aedes trap (BG-GAT® trap from Biogents). During 3 years from 2021 to 2023, the effectiveness of 3 phases were assessed: Trapping only in year 1; Trapping and door-to-door breeding sites suppression in year 2; autonomy of the strategy (traps managed by inhabitants) in year 3. The entomological monitoring consisted in fortnightly captures using BG-Sentinel® traps in intervention and control areas, and a sociological survey was carried out focusing on the behaviour and perception of the residents from intervention and control areas. Our results tend to show that the use of gravid aedes traps has an effect when this strategy is combined with door-to-door and control of breeding sites. In addition, a regular monitoring of the passive gravid traps seems essential to guarantee the trapping network efficiency. The ultimate goal of the project, implemented for 3 years, is to demonstrate the effectiveness of this mass trapping method and identify key factors that could maximise the adult mosquito population reduction, in order to provide municipalities wishing to implement urban mosquito control programmes locally with an acceptable and cost-effective strategy.

Field efficacy of consecutive VectoMax® FG larvicide treatments against *Aedes albopictus* and *Culex pipiens*

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Since its appearance in 2003, the exotic invasive tiger mosquito, *Aedes albopictus*, has been under constant surveillance in southern Switzerland. To control its population, an integrated vector management strategy has been implemented. In public areas, the focus is on the aquatic phase of the mosquito's life cycle, involving the removal of breeding sites and the application of larvicides, primarily in catch basins. One effective larvicide used is VectoMax® FG, developed by Valent Biosciences. It combines *Bacillus thuringiensis var. israelensis* (strain AM65-52) and *Bacillus sphaericus* (strain ABTS-1743). Notably, this biological mosquito larvicide exhibits long efficacy, lasting over a month, which reduces the need for frequent applications and minimises the required quantity of product and personnel. In 2021, we evaluated the effectiveness of VectoMax® FG against *Aedes albopictus* and *Culex pipiens* in real urban catch basin conditions within southern Switzerland. Employing singular treatments at several sites during the mosquito activity season, we sought to understand its efficacy over time from the treatment's initial moment. Building on this research, we continued our efforts in 2022 by repeating the experiment, this time applying four (i.e. every six weeks) or three (i.e. every eight weeks) successive treatments during the mosquito activity season. This investigation aimed to unveil any potential cumulative effects of the larvicide, confirm the results from 2021 and provide comprehensive guidelines to local authorities responsible for its application.

Seasonality, ecology and control of floodwater mosquitoes in a summer flooded riverine wetland in Britain

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Aedes vexans is a rare mosquito in the United Kingdom, known to occur at a small number of locations in low numbers. In 2018, UKHSA Medical Entomology responded to an enquiry from Bassetlaw District Council regarding extreme nuisance biting, and by running an adult mosquito trap (Mosquito Magnet), identified *Aedes vexans* as the dominant species, a known vector of Rift Valley and Tahyna viruses. This study aims to develop ecological understanding of Aedes vexans, a species rarely recorded in the United Kingdom, in a floodplain wetland along the River Idle, Gamston, Nottinghamshire, United Kingdom. Habitat mapping was conducted across the study site, and weekly mosquito larval surveys conducted using a standard dipper at 18 sites representing 6 habitat types: river, open water, flooded grassland, swamp, wet woodland, and reedbed. Additional variables including water temperature, water depth, number of weeks since drying, vegetation height, application of larval control products and vegetation cover were recorded at each sampling location. Larval data was passed to the local authority in order to identify habitats requiring larval control, and larval control conducted using two licensed products in the United Kingdom for mosquito control: a BTI product (Vectoac-12AS) and a PDMS (Aquatain mosquito film). *Aedes vexans* was the most abundant larvae, and was first reported in week 19 (14th May 2021), last reported in week 41 (22nd October 2021), with highest abundances in week 25. Other *Aedes* floodwater species were also present: *Ae. caspius*, *Ae. sticticus*, and *Ae. cinereus*. Additional species included *Anopheles maculipennis*, *Culex* pipiens s.l., *Ae. cantans* and *Ae. flavescens*. There were strong relationships between the presence of *Aedes vexans* and habitat type and the number of weeks since drying, with *Ae. vexans* found predominately in swamp and flooded grassland, between 1–3 weeks since rewetting.

An update on the ecology, seasonality and distribution of *Culex modestus* in England

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Culex (*Barraudius*) *modestus* (Ficalbi, 1980) is an important vector of WNV (West Nile virus) in Europe and it has the potential to have a play an important bridge vector role in future WNV transmission in the United Kingdom. Here we provide an update on the known distribution of the species in England based on adult and larval data, characterise the preferred *Cx. modestus* larval habitats, and present adult and larval data from sites where the species is known to occur. *Culex modestus* is primarily found in the south-east of England, particularly in North Kent, the Thames Estuary, and along the Essex coast, and has been found as far east as Sandwich, Kent. Adult numbers peak in mid-late July, with larval numbers highest in late August/early September. Preferred habitats in North Kent are warm, shallow, narrow ditches, with an abundance of marginal, submerged, and floating vegetation. Such data on the distribution, seasonality and habitat preference of *Cx. modestus* are critical in informing WNV surveillance programmes, identifying at risk areas (associated with this species) and providing information for a targeted control strategy in the event of disease transmission.

Vector control in a Spanish natural environment, La Peña reservoir

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Mosquito control can be a relatively simple task when conditions allow it. However, what happens when it comes to natural environments or protected areas where environmental legislation regulates access? The implementation of a sustainable control plan adapted to each place and at each time allows treatments to be optimised, thereby achieving the desired success. The La Peña reservoir regulates the water flow of the Gállego river (Huesca, Spain), in the Aragonese Pyrenees, and is a clear example of how the changing conditions of an enclave can impact the density of mosquitoes at any given time. Therefore, the volume of impounded water is affected each year not only by environmental conditions, but also because of the water demand for various activities such as agricultural and recreational activities in the Gállego environment. All these factors produce fluctuations in the water level at the beginning of spring and during the summer, increasing the proliferation of mosquitoes in the area. Moreover, the abundant vegetation present on its banks provides an optimal environment for the breeding and shelter of these insects, entailing a difficulty to be faced in control work. In the absence of adequate control, mosquito densities in La Peña reach high values, causing significant inconvenience in the surrounding municipalities. In this context, since 2020 Quimera Biological Systems S.L. has been developing and implementing a control plan which takes into account the variability of this environment and adapts to each temporary circumstances. A good knowledge of the reservoir geography has been essential in order to identify possible breeding sites, including permanently flooded spots ideal for Culex mosquitoes and areas susceptible to flooding, perfect for the biology of the Aedes genus. Furthermore, given the impossibility of carrying out adulticidal treatments, the key strategy has been the elimination of larval stages with *Bti* (*Bacillus thuringiensis var. israelensis*), as well as monitorisation of immature and adult stages. The results obtained in these assessments show an evident dependence between the reservoir level and mosquito larvae densities. Moreover, it has been observed that different mosquito species present in La Peña accelerate their biological cycle as a consequence of the stress generated by these changing factors, as well as the high larval densities, therefore influencing the frequency of treatments. Our work presents some results of several years of the mosquito monitoring and control programme carried out in the surroundings of the La Peña reservoir.

Effect of water salinity on immature performance and the lifespan of adult Asian tiger mosquito

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Aedes albopictus (Skuse, 1894) is a vector for pathogens like dengue, chikungunya and Zika viruses. Its adaptive capacity enables reproduction in temperate climates and development in artificial containers with freshwater in urbanised areas. Global warming and the consequent rise in sea levels will increase saline and brackish water in coastal regions. This adaptation to more saline environments may facilitate the expansion and establishment of *Ae. albopictus* in new areas, with the threat this poses to public health. The influence of salinity on the survival of larvae and adults of *Ae. albopictus*, previously considered toxic and sometimes used as a low-cost method to control freshwater mosquito larvae, was investigated under controlled laboratory conditions. First instar larvae were exposed to different salinity concentrations (0 to 30 ppt) and their development time and survival were monitored daily. We used Kaplan-Meier and Cox regression models to analyse the survival rates at different salinity levels. Increasing salt concentrations significantly elevated the mortality risk during immature development ($P<0.0001$), while no significant effect was observed on adult mortality risk ($P=0.19$). A comparison between distilled and bottled water revealed a notable increase in overall mortality risk for individuals developing in distilled water ($P<0.0001$). However, no significant effects were found when analysing survival from the first larval stage to adult emergence ($P=0.672$) and adult lifespan ($P=0.193$). Our findings suggest that *Ae. albopictus*, previously considered freshwater species, can successfully develop and survive in brackish waters, even in the absence of characteristic structures found in euryhaline species. These adaptations may enable *Ae. albopictus* to establish new breeding sites and colonise unexplored territories. Fluctuations in temperature and climate are expected to activate new potential habitats, influencing vector dispersal patterns and introducing changes in disease transmission risks. An in-depth study of potential new habitats is key to integrated control and prevention of disease risks and transmission affecting human and animal health.

Wolbachia pipientis infections in populations of *Aedes albopictus*, *Culex pipiens* and *Drosophila melanogaster*

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Since the first report of the presence of *Aedes albopictus* in Valencia, innovative tools had been developed for its control. One of the strategies involves the endosymbiont *Wolbachia*, a bacterium that has been shown to interfere with the reproduction of this species through a phenomenon called cytoplasmic incompatibility (CI). This is caused by the existence of male mosquitoes infected with incompatible strains to those harboured by the females. As a first step before implementing a large-scale Incompatible Insect Technique (IIT) control, we characterised the *Wolbachia* strains naturally-infecting *Ae. albopictus* and *Cx. pipiens* in Valencia city and *Drosophila melanogaster* in Requena (Valencia). Eggs from the two mosquito species were collected from the 19 districts of the city and reared to adults. In parallel, *D. melanogaster* was collected from Requena and the resulting isolines were maintained in the laboratory. The first part of our study revealed that 94% of the *Ae. albopictus* analysed individuals were naturally infected with *Wolbachia* wAlbA and wAlbB, with most samples carrying co-infections. As for the characterisation of the *Wolbachia* strains infecting autochthonous *Cx. pipiens* and *Drosophila*, all *Cx. pipiens* individuals characterized seem to be infected by a single strain, while the Requena populations of *Drosophila* seem to carry two strains. The CI value in *Ae. albopictus* of these three strains needs to be determined by microinjecting them into *Wolbachia*-free *Ae. albopictus* maintained in our laboratory. These data are the first characterisation of *Wolbachia* strains in the territory of the Comunitat Valenciana and represent a major opportunity for the possible implementation of a biological control strategy.

Mosquito breeding in street catch basins: diversity of species and effects of a residual treatment in Valencia

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Street catch basins are known to be frequent breeding and adult resting sites for mosquitoes. *Aedes albopictus* is an invasive species currently widespread in many countries of southern Europe while *Culex pipiens* is a common autochthonous species. These species' potential role as disease vectors poses a public and veterinary health threat and control activities are conducted, including larvicide treatments in public catch basins. We aimed to investigate the presence and species diversity of immature stages in street catch basins from the residential area of El Vedat de Torrent, located near the metropolitan area of Valencia in eastern Spain. We also studied the impact of the treatment with an insecticide paint applied on the interior sides of the catch basins. This study belongs to the project NESCOTIGER, which aims to develop a novel strategy for the control of tiger mosquitoes in residential areas. We randomly inspected catch basins in two rounds during August and October 2022, a dry and rainy period, respectively. Water samples were collected and divided into two 150-ml aliquots for adult emergence studies and species identification. We inspected nearly 200 catch basins in all. We found that water presence was lower in August (18; 36%) than in October (26; 51.5%), a period characterised by the autumn rainfalls. From those water-containing catch basins, we less frequently found immature stages in August (11; 61.1%) than in October (18; 70.6%), with a total of 227 *Cx. pipiens* and 28 *Ae. albopictus* larvae identified. Catch basins were infested by only *Cx. pipiens* (47.4%), *Ae. albopictus* (21.1%), or by both species (31.5%). Also, we recorded different adult-emergence proportions between the two surveys, with a preponderance of *Ae. albopictus* (31 and 25) versus *Cx. pipiens* (4 and 16) in August and October, respectively. Street catch basins of the control area showed similar water presence percentage (46.2%) but higher positivity (91.7%) and dominance of emerged *Cx. pipiens* (14) versus *Ae. albopictus* (8). Total mosquito emergence was 17.3% and 53.3% for treated and control basins, respectively. Overall, public street catch basins in the El Vedat de Torrent residential area were confirmed as *Cx. pipiens* and *Ae. albopictus* breeding sites. Treatment with the insecticide paint reduced positivity and adult emergence ratio of both species. Further studies are necessary to fully understand the oviposition behaviour of these mosquitoes in the catch basins and the specific effects of the insecticide paint.

Monitoring the effects of mosquito control on the biodiversity of wetlands

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Nowadays, only *Bacillus thuringiensis var. israelensis* (*Bti*) is duly authorised to control breeding sites of mosquito species along the French Mediterranean coast. Despite its specificity, *Bti* treatments may cause some side effects in other non-target dipteran species such as chironomids. The indirect impact on aquatic and terrestrial food chains should therefore be evaluated. In the presented work, an environmental DNA approach is used for the monitoring of aquatic fauna and to investigate mosquitoes and non-target organisms ratio in birds diet. Captures of bird individuals and their faeces analysis, along with the use of insect traps have been carried out before and after *Bti* treatments in wetlands during the period of August and September 2022. As a control, birds captures and mosquito traps were also performed in a similar natural site. In total, considering all sites and samples, a minimum of 286 species have been detected in bird's diet. Overall, the main orders consumed were: Diptera>Coleoptera>Odonata>Araneae>Lepidoptera. Although Culicidae family is present in the diet of all bird species assessed in this study, only 25.1% of the captured bird individuals had consumed mosquitoes, with *Aedes caspius* being the principal specie consumed. The short period of this study and the relatively small sample size did not allowed to get a clear answer to the potential impact of *Bti*. Nevertheless, these initial results, together with a study on the diet of four reedbed passerine species (http://hdl.handle.net/2268.2/12600) which was recently carried out in the frame of the Occitania Coastal Reedbeds Project (https://www.roselieres-occitanie.fr/), prove the usefulness of new molecular techniques as a tool to assess the potential unintentional effect of mosquito control on non-target species inhabiting French wetland reedbeds.

Mosquitoes and rewetting of peatlands

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Rewetting of drainage ditched wetlands have recently been acknowledged as a good way to reduce emission of greenhouse gasses. Especially peat soils are targeted when climate focused rewetting is under subject. In association with rewetting projects people living nearby have had concerns and fear of mosquito nuisance problems. During 2022, a field study as well as a literature review was performed. The mosquito fauna in nutrient poor peatlands, close to the river Dalälven basin where *Aedes sticticus* is a big nuisance problem, were studied with a combination of methods. During spring, larvae were collected. During the summer, traps were used to catch flying mosquitoes. From the nutrient poor peatlands only *Aedes punctor*, *Aedes annulipes* complex and *Culiseta morsitans* hatched, while in the surrounding wet woodlands *Aedes cinereus*, *Aedes communis* and *Aedes diantaeus* hatched, indicating that the acidic condition of the peat is unfavourable for many mosquito species. During the summer traps were placed on the peat bog and in the collections of blood seeking mosquitoes more species are present, including *Aedes sticticus* in low numbers. Together with a review of earlier literature we conclude that the nutrient poor peatlands themselves are not favourable for most mosquito species and will not increase nuisance caused by *Aedes sticticus* but constitutes a cool and humid environment for many mosquitoes during summer. During 2023, 6 wet areas with nutrient rich peat soil were studied. Two were drained by ditches (but suggested for rewetting by authorities), three were rewetted in the last 2–4 years and one had not been drained. All areas were investigated with collections of larvae and traps. In order to assess the spread of mosquitoes from rewetted areas, larvae were collected, and CDC traps were positioned in two transects 100, 200 and 300 metres out from the centre. One transect was in more open areas and the other in forest. The results of this year's studies have not yet been compiled but will be presented at the conference. There were fewer larvae collected in ditch drained areas compared to the rewetted and naturally wet areas. Also in these collections we find a wider range of mosquito species in trap collections compared to collected larvae. Overall, we have noticed a wider range of species in the more nutrient rich peat soil habitats, compared to the nutrient poor peat lands studied 2022.

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ORAL PRESENTATIONS

Combining SIT with automation and machine learning to reduce mosquito-borne disease globally

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The Debug project aims to control wild populations of the mosquitoes *Aedes aegypti* and *Culex quinquefasciatus*, which are the main vectors of diseases such as dengue, Zika, chikungunya, West Nile and avian malaria. Traditional mosquito control methods, such as source reduction and chemical insecticides, have proven inadequate in controlling these species and new tools are urgently needed. Releasing sterile male insects that mate with wild females to generate infertile eggs and thus reduce mosquito populations is a promising alternative strategy for mosquito control. The sterility of males can be induced by irradiation or by incompatible crosses between *Wolbachia*-infected male mosquitoes and uninfected wild females, (known as the incompatible insect technique (IIT)). However, implementing SIT in mosquitoes presents unique challenges, such as male quality, mosquito mass production and distribution. Debug has developed proprietary technology that automates the rearing, sorting, and release of millions of non-biting, non-GMO, sterile male mosquitoes at scale. The end-to-end technology includes automated rearing robots that takes first instar larvae as input and outputs uniformly sized pupae, AI powered visual sex sorting systems, and data surveillance and release platforms to optimise efficiency and performance. We have partnered with public health, private sector, and vector control agencies worldwide to increase the availability of our technologies that target disease vectors and reduce their impact. We have successfully conducted a series of field programmes worldwide, including California and Australia, where *Ae. aegypti* populations were reduced by >95% and >80%, respectively, compared to similar areas without releases. We have also been working with the National Environmental Agency in Singapore on an incompatible *Ae. aegypti* male programme since 2018, achieving up to 98% suppression of biting female mosquitoes and a corresponding 88% reduction in dengue cases compared to control sites. We slated to launch our first *Culex quinquefasciatus* field programme later this year in Maui. Communities and environments affected by mosquito borne diseases are in dire need of additional tools to protect human and animal populations from existing and novel pathogens. Debug's automated rearing and release technologies are flexible and can support any approach that requires mass production of mosquitoes.

Application of SIT as an integrated control measure for *Aedes albopictus* in the Canton of Ticino (Switzerland)

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The exotic invasive tiger mosquito, *Aedes albopictus*, appeared in Canton Ticino (southern Switzerland) in 2003. The spread of the mosquito has been surveyed constantly since then, and an integrated vector management, focused on the juvenile stages of the insect, has been implemented to control its numbers. Despite the system has proven its ability to contain tiger mosquitoes the densities in the territory of *Ae. albopictus* still present a risk factor for disease transmission. In addition, there is a growing practice by citizens to utilise automatic dissemination systems of products against mosquito adults thus running the risk of facilitating resistance processes. The aim of this project is to test whether the application of the Sterile Insect Technique (SIT) can bring these densities below the risk threshold, reduce the nuisance at the climatic and urban conditions present in the region and give an answer to the citizens. SUPSI is a partner without funding of a WHO-IAEA (TDR Sterile Insect Technology Project in partnership with CDC, IAEA and NTD/WHO) global project that is precisely about testing whether the application of SIT can effectively and sustainably reduce the incidence of diseases related to *Ae. albopictus* and *Ae. aegypti*, such as dengue, chikungunya and Zika. The field outcomes collected in the Canton Ticino setting will therefore be made available to the wide vector control community to contribute to the overall evaluation of this technique when applied to invasive *Aedes*.

VEClim: an early warning support system for climate-sensitive vector-borne diseases

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Global warming, due to human-made greenhouse gas emissions, affects biodiversity, species phenology and ranges, and ecosystem processes. Many insect species that are disease vectors, including the pathogens they transmit, are exposed to these global trends, posing challenges to human and environmental health. To plan effective control strategies, early warning and decision support systems are urgently needed. The Climate-Driven Vector-Borne Disease Risk Assessment project (VEClim), supported by the Wellcome Trust as part of Digital Technology Development Awards in Climate-Sensitive Infectious Disease Modelling, aims to improve vector-borne disease prediction and management by employing data-driven, mechanistic, and climate-sensitive geographical modelling to represent vector populations and disease transmission. The VEClim platform hosts mathematical models, incorporating physiological processes driven by climate and environmental factors. A user-friendly web-based GIS is designed as a versatile interface to improve accessibility to the models and to present short-, medium-, and long-term predictions of habitat suitability, vector activity, and disease risk and impact. The platform displays vector presence and risk maps and seasonal profiles of vector activity and disease risk. It is planned to include: 1. an extensive database of meteorological variables, climate projections, and environmental covariates; and 2. an up-to-date longitudinal vector surveillance dataset. Customised simulations under different climate scenarios and vector control activities will also be possible. A comprehensive application programming interface (API) will extend the capacity of advanced data analysis tools, such as Python and R, to accommodate climate-sensitive mechanistic modelling. The platform is operationally maintained at The Cyprus Institute and is permanently available via its dedicated domain: veclim.com. Through an active user community and maintaining communication with citizens, public health specialists, and fellow researchers, we prioritise identifying needs and designing for better utility and accessibility. We advocate predictive tools to reduce the environmental impact of insecticides due to better timing and higher efficiency of vector control and to prevent disease outbreaks due to the incorporation of climate-sensitive risk assessments into early warning systems.

A mechanistic model to predict spatial temporal patterns of *Culex pipiens* s.s./*Cx. torrentium* in Germany

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Mosquitoes are well known for their ability to transmit pathogens. These include a variety of arthropod-borne viruses (arboviruses) of medical and veterinary interest. Due to globalisation and climate warming, the threat of (re)emerging arboviruses is increasing in Europe. This also applies to temperate regions, where the transmission of viruses is becoming possible due to an increase in ambient temperature, i.e. shortening the extrinsic incubation period. *Culex pipiens* s.s. and *Culex torrentium*, commonly found in and around human settlements, are the primary vectors of Usutu virus and West Nile virus in Germany. The prediction of the spatial-temporal occurrence of these mosquito species is needed for the assessment of arbovirus transmission risk and to timely organise intervention methods, such as vector control. On the basis of a pre-existing model, a mechanistic model was developed to predict the spatial-temporal occurrence of *Cx. pipiens* s.s./*Cx. torrentium* in Germany. The model output is driven by local rainfall and temperature data downloaded from the Open Data Server of the German Meteorological Service. In a nation-wide field study in 2021, population data on *Cx. pipiens* s.s./*Cx. torrentium* was collected and used to evaluate the model prediction. This evaluated mechanistic model can be used to simulate vector control measurements or the impact of increasing temperatures in cause of climate warming.

MosChito rafts: eco-compatible floating structures for the control of larvae of native and invasive mosquitoes

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Biolarvicides are effective and eco-compatible tools for mosquito control, and formulations based on *Bacillus thuringiensis israelensis* (*Bti*) are widely used in the domestic environments and in municipal pest control programmes. However, since *Bti* has low residual activity and requires repeated applications, the development of products that provide prolonged delivery and efficacy of *Bti* is a highly desirable. To this purpose, we developed MosChito raft, a novel type of hydrogel-based structures, suitable to incorporate biolarvicides. The goal was to generate floating structures for the release of bioinsecticides, with the following characteristics: eco-compatibility, attractiveness for mosquito larvae and a protective action on *Bti*, with prolonged delivery of this bioinsecticide. The efficacy of MosChito raft was tested on larvae from both native and alien mosquitoes, and its eco-compatibility was then validated on two non-target model organisms. The prototype MosChito raft, consists of a hydrogel of chitosan crosslinked with genipin, containing yeast cells as attractants for larvae and a *Bti*-based larvicide. Mortality and attractiveness bioassays were performed on larvae of different mosquito species (*Aedes albopictus*, *Culex pipiens* and *Aedes koreicus*). Firstly, the efficacy of the MosChito rafts was tested under laboratory conditions, and then under semifield conditions. Thereafter, the nontoxicity of MosChito raft was observed on two non-target aquatic organisms used as laboratory models for ecotoxicity analyses. Mortality, development, and motility tests were performed on these organisms, under prolonged exposure to MosChito raft. The results showed that MosChito rafts possess larvicidal capability against larvae of all tested mosquito species, with a slightly different timing of action, due to the different behavioural habits of larvae. In addition, no evidence for toxicity was recorded on non-target organisms, which posit MosChito raft as an eco-compatible product, potentially suitable for a wide use. In summary, MosChito raft holds the potential to be applied as a novel tool for the delivery of *Bti* to larvae of different mosquito species, suitable to prolong the efficacy of the bioinsecticide and to reduce the doses needed to achieve an effective control.

Targeting host factors to stop viral replication in *Aedes albopictus*

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Mosquitoes are key hosts for replication and dispersal of arthropod borne-viruses (arboviruses) which are a continuous threat to public health causing approx. 70 000 annual deaths. Arboviruses exploit in animals' cell components such as furin for cell cleavage and entry through the cell membrane. In previous studies, Furin inhibition showed active antiviral properties and successfully stopped arbovirus replication in mammalian cells. However, inhibition of furin and its role on mosquitoes and insect cells remain unknown. Here we report the effects of selected peptidomimetic furin inhibitors on insect cells, in different mosquito life stages and their antiviral efficacy to decelerate Semliki Forest virus (SFV) replication. The selected inhibitors had no toxic effect on the C6/36 and U4.4 cell lines, but the inhibitors MI-1148 and MI-1554 were toxic to several mosquito life stages, while MI-1851 had no negative effect. Finally, all selected inhibitors successfully reduced viral replication of SFV in cell culture. Furin inhibition is a promising strategy to target arboviruses, offering new ways for vector control.

Preliminary results on the implementation of a boosted Sterile Insect Technique targeting *Aedes albopictus*

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Aedes albopictus is an invasive mosquito species (IMS) of great concern for public health, due to its severe nuisance and its vectorial capacity for many diseases such as dengue, chikungunya and Zika viruses. Its management is complex and requires a combination of several control tools. A pilot project for the integrated management of *Ae. albopictus* is being implemented in different European countries (France, Greece, Spain) including the application of the boosted Sterile Insect Technique (boosted SIT). One of the project actions was to field-test the efficacy of releasing irradiated *Ae. albopictus* males impregnated with a pyriproxyfen based powder (CAS No. 95737-68-1). The results of boosted sterilised males' releases revealed a significant reduction of both egg hatchability and mosquito density in target areas where the technique was applied in comparison to control areas. These promising results constitute a significant advance, proposing the boosted-SIT approach as a new component of integrated management of the Asian tiger mosquito in line with EU Regulation 528/2012. This will also encourage European countries to prepare a management plan for the control of new IMS (e.g. *Ae. aegypti*) in the event of a possible invasion (management of imported IMS population at entry points).

E4Warning: preliminary data on mark release recapture of *Culex pipiens* in Switzerland

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Early detection and prevention of disease outbreaks is a key pillar in any preventive control strategy. In the case of mosquito-borne diseases (MBDs), a crucial component of risk assessment is to gain knowledge on vector and host distributions, their ecological interactions and their modulation through human activity and environmental conditions. Quantifying and uncovering human-vector-reservoir interactions and transmission pathways has been challenging, but important gaps remain. E4Warning Horizon Europe Research and Innovation Action (Grant Agreement 101086640) will go beyond the state of the art by introducing innovative vector and host monitoring tools that can harness the current data revolution with the sole purpose of public health preparedness. The aim is to develop an eco-epidemiological intelligence for early warning and response to mosquito-borne diseases risk in endemic and emergency settings. The consortium is formed by 12 institutions from six European countries (Spain, Germany, Greece, Belgium, Switzerland, and the United Kingdom), which are contributing on diverse expertise in entomology, spatial ecology, epidemiology, earth observation science, sensor engineering, sociodemography, and spatial statistic modelling. Among the project's aims is to understand the temporal dynamics in transmission probabilities of MBDs. Since birds are the main reservoirs of mosquito-borne pathogens (e.g. West Nile – WNV and Usutu - USUV viruses) and responsible for the long-distance dispersal of other zoonotic diseases, by applying models of bird species composition and their seasonal dynamic in relation to mosquito dispersal, will enable to assess the seasonal probability of MBDs transmission from migrating species to local species, between local species, and to humans. We here describe the preliminary data of a trial on the dispersal of *Culex pipiens*, the mosquito species responsible for WNV and USUV transmission, using a mark-release-recapture (MRR) technique. Fluorescent-marked *Culex pipiens* originated from eggs, larvae and pupae collected in the field were released in a natural wild area bordering with a village in Canton Ticino (southern Switzerland) during the summer of 2023. Adult traps, deployed at 50, 100 and 150 metres from the release point, were run for three-four days post-releasing and fluorescent recaptured mosquitoes were reported.

The value of data collection and processing in mosquito control projects

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Data collection is a crucial component of mosquito control projects, as it plays a significant role in mosquito control projects by providing the necessary information to target interventions, monitor mosquito populations, prevent disease outbreaks, and ensure the efficient use of resources. The 'ebite' online platform is a unique and innovative suite of flexible digital tools for recording, monitoring, visualising and analysing the actions performed and the data collected in the field in real time for large-scale mosquito control projects (patented). The architecture of eBite includes the 261 000 digitised geo-referenced breeding sites in the wetlands, agricultural, suburban, and urban system (in 5939 settlements in 112 Municipalities out of a total of 300 all over Greece). 12 677 000 records from 288 000 field inspections made by the 130 technicians of Ecodevelopment in the field, have been registered in 2023. The five-year database of eBite platform (2018–2022) were used to derive several key indices and metrics for comparison between previous years and 2023, to assess the effectiveness of control efforts and monitor changes in mosquito populations and WNV transmission. Some of the key indices that were used in mosquito control projects in 2023 were: 1. Adult populations of Aedes spp. and Culex spp. in the city of Thessaloniki, in the periurban system, in the wetlands and in the rice fields; 2. WNV pathogen circulation, by tracking the prevalence of WNV in *Culex* populations and in sentinels (chickens); 3. Density of mosquito larvae (*Aedes* spp. and *Culex* spp.) in breeding sites; 4. Critical observational data 5. Environmental data, mainly rainfalls, temperature and degree-days The regular data collection on larvae populations and on the characteristics of the breeding sites after every inspection, using the eBite platform, enables us to produce a prediction model for the presence of mosquito larvae at the breeding sites on a weekly basis (BoL). The recall of this model is 80% for the presence of *Culex* larvae and 90% for the absence of *Culex* larvae. Data collection provides the evidence needed to make decisions about which control methods to use, where to allocate resources, and when to implement interventions. By comparing the key indices over time, mosquito control programmes can make informed decisions, adjust strategies, and demonstrate the effectiveness of the efforts in reducing mosquito populations and mitigating the risks associated with mosquito-borne diseases.

Session 8. New technologies and practices in vector control

POSTERS

Optical recognition of the eggs of four Aedine mosquito species

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Invasive mosquitoes have been a public health hazard to temperate regions for the past 30 years. In continental Europe, surveillance data is not reliable due to the simultaneous presence of three main invasive (*Aedes albopictus*, *Ae. japonicus* and *Ae. koreicus*) and a native (*Ae. geniculatus*) species. Current techniques, such as ovitraps and molecular biology (PCR and MALDI-TOF MS), fail to measure the density of invasive species and detect early new invaders in a short time and at low cost. Here, we optically examined Aedine eggs with a high-resolution stereomicroscope and determined the species by MALDI-TOF MS. In this way, we looked for specific characters in eggs of different species that could easily lead to their determination. To validate the methodology, we then created 60 questionnaires that were distributed among experts and non to experts in entomology during two tests. Our aim was to test whether, with initial training, certain species could be easily identified by looking at the pattern on their exochorionic membrane. To evaluate the performance, sensitivity and specificity, among other measures of accuracy, were calculated. Both tests showed consistent results: raters did not have difficulties in determining *Ae. albopictus* and *Ae. geniculatus*, while they had more difficulties in distinguishing *Ae. japonicus* from *Ae. koreicus*. In general, the quality of the exochorion seemed to play a more important role than the level of the rater. It is a significant achievement to be able to differentiate *Ae. albopictus* from the other two invasive species, as it is currently the most problematic species at the public health level in Europe. Due to the presence of other species that might prevent the correct quantification of mosquito population densities using standard identification methods and due to *Ae. aegypti* threat, it is recommended to optically determine also other species.

Random mutations induced by a sub-sterilising dose GR on *Ae. albopictus* male pupae and transmission to progeny

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The Sterile Insect Technique (SIT), used for controlling mosquitoes, involves breeding, separating sexes, sterilising males with radiation, and then releasing them. However, high doses of gamma radiation that ensure male sterility also damage them, reducing their ability to compete for mates with wild males. Thus, it's preferable to use radiation doses that cause sub-sterility. Males treated with these sub-sterilising doses still produce some offspring, which might carry sub-lethal random mutations that could pass on to the next generations. To study this, we exposed male *Aedes albopictus* mosquito pupae to sub-sterilising gamma radiation (2–4% egg hatching rate) and explored expressed mutated genes in both treated males and their offspring using RNA-seq. We identified single nucleotide polymorphisms (SNPs) using two methods, only considering those identified by both (less than 5% of total predicted SNPs), and annotated them to genes. We found over 600 genes with mutations likely caused by radiation in treated male mosquitoes. This suggests that gamma-ray treatment may lead to broader genetic variations than just dominant lethal mutations. Some of these mutated genes were also present in the male offspring of the first and second generations, suggesting that radiation-induced genetic changes can be inherited. Analysing the mutated genes with g:Profiler, we found minimal impact on biological processes, except for one instance (oxidative phosphorylation). Only in a few cases (oxidative phosphorylation, UDP-glucose metabolic process, proton transmembrane transport, and riboflavin metabolism) did we observe significant effects on biological processes in the male offspring with transmitted mutated genes.

Pilot study to evaluate alternative and eco-friendly methods for mosquito control in an urban area (Italy)

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Mosquito control is one of the main preventive measures of mosquito-borne diseases transmission. In this study, we present the results of a pilot study where the use of a biodegradable larvicide, communication campaigns and citizen involvement have been implemented. The study was carried out in the summers of 2021 and 2022 in a village of Northeast Italy (Ponte di Piave, Veneto Region). In this site, a selected area (treated area) was applied a vegetal oil with physical-mechanical action in all catch basins located in public areas while in another area (control area) catch basins were treated with Diflubenzuron and VectoMax® FG. In the treated area, door-to-door visits were conducted to inform population and provide information material and larvicide for free. Meetings were held with the population to inform them of the aims of the project. To monitor the efficacy of the two control methods, 47 out 350 catch basins were inspected 10–14 days after larvicide application to assess larvae presence; in addition, eight ovitraps were placed both in the treated and in the control area. In 2021, the average of positive catch basins in treated area increased of 39.3% while decreased of 63.7% in control area. The average number of eggs per collection was higher in treated area (257.3 eggs/ovitrap) compared to control area (212.5 eggs/ovitrap). Due to these results, in 2022, the vegetal oil has been applied more frequently and at twice dosage than that recommended by the company. In 2022, the average of positive catch basins decreased after treatments in both the treated and control area of 51.5% and 87.3%, respectively. In 2022, the average number of eggs per collection was lower in the treated area than in the control area (139.1 eggs/ovitrap vs 172.2 eggs/ovitrap). In conclusion, the vegetable oil-based larvicide was not effective in public area applications due to the low persistence in catch basins. For the public administration, its cost for application is higher than the traditional larvicide use. In private areas, its use is recommended, since it is safe for citizens, who can frequently apply it (weekly) in non-removable larval breeding sites. However, in 2022, the implementation of communication campaigns resulted in fewer mosquito numbers (meaning as eggs/ovitrap) in the treated area than in the control. This work was carried out in the frame of the CCM Project 'Preventing vector-borne diseases through the development and pilot implementation of new operational supporting tools'.

RNA interference as an alternative control strategy against mosquito populations

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Mosquitoes are a nuisance and of major medical importance. According to WHO, mosquitoes are considered the most dangerous animals because of the negative impact of the pathogens they transmit. For a long time, the risk of mosquito-borne diseases was restricted to tropical and subtropical regions. But due to climate change, land use changes, and urbanisation, especially species of the genus *Aedes* have expanded their distribution range to more temperate regions. A lot of the available control measures have negative effects on non-target organisms, animals, humans, and the environment. RNA interference (RNAi) a post-transcriptional gene silencing mechanism mediated by RNA molecules (e.g. dsRNA, siRNA, and miRNA), is a promising novel insect control method. This mechanism allows species-specific control of mosquito populations, without posing any negative effects on non-target organisms. There are several studies on possible target genes in many pest insects e.g. *Tribolium castaneum*, *Drosophila suzukii*, or *Aedes aegypti*. However, studies on one of the major vector species *Aedes albopictus* are scarce, thus in this work, we present a fundamental study to identify possible target genes for the control of *Ae. albopictus* using dsRNA to induce RNAi response. We also verified the potential of different gene targets in first cell culture tests using U4.4 as the major cell line. As the C6/36 cell line is suspected of dysfunctional RNAi response, we decided to use this cell line as a control to determine if the selected dsRNA constructs have similar RNAi-mediated effects. Our results showed no significant effect in C6/36 cells when treated with dsRNA of different target genes. Whereas the treatment of U4.4 cells showed significant reductions in cell viability especially when applied in complex with commercially available transfection reagents. Out of the selected genes, we observed the highest reduction of cell viability ranging between 70–40% in the cells treated with 2.5–0.5 µg naked dsRNA/well of IAP2. The cell viability is further reduced by over 80% when treated with 0.2 µg complexed dsRNA/well. Therefore, we suggest using U4.4 cells can be a cost- and time-effective first step in determining possible target genes for *Ae. albopictus*.

Validation of FTA cards to detect mosquitoes and arboviruses in surveillance programmes

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The pathogen surveillance of field-sampled mosquitoes is time-consuming and costly as it usually involves the screening of large number of samples collected by e.g. CO_2–baited traps. To reduce the effort, mosquito traps can be equipped with honey-baited Flinders Technology Associated (FTA) cards. The trapped mosquitoes fed on the FTA cards directly in the field, whereby they inject their saliva and possibly viral RNA, which is preserved on the FTA cards at room temperature without degradation. This study aims to investigate the sensitivity of the FTA cards for arboviruses and mosquito species detection. Furthermore, we analised how long FTA cards are attractive for mosquitoes in dependence of different sugar-based solutions. The sensitivity was studied for Sindbis virus (SINV) via dilution series applied to FTA cards. The virus RNA was extracted from each FTA card and screened by a Pan-Alpha PCR and by a RT-qPCR. The time period of the attractiveness of FTA cards for mosquito feeding was studied by means of cage experiments with *Cx. pipiens* biotype *molestus* and *Ae. albopictus*. FTA cards were humidified with either cotton pads or a newly developed system. Three different sugar-based solutions (fructose solution, artificial honey and artificial honey with 2% hydroxy-ethyl-cellulose) were dried 1, 2, 4 and 10 days and subsequently offered to the mosquitoes. For the direct detection of mosquito species, different numbers of *Culex* mosquitoes were allowed to feed on FTA cards. Mosquito species DNA was extracted from FTA cards and a multiplex *Culex* Taxo qPCR was performed. We could show that the RT-qPCR was more sensitive compared to the Pan-alpha PCR. Comparing virus extraction from the virus stock with extractions from FTA cards yielding the same amount of identical stock revealed that the application onto FTA cards leads to a substantial drop in the amount of RNA being detected. This might lead to false negative results. Therefore, the detection limit is currently under investigation. With a new developed humidification system for FTA cards, we generated high feeding rates (80–100%) for *Ae. albopictus* and *Cx. pipiens* biotype *molestus* even after ten days of desiccation. We observed no significant difference in the attractiveness of the three tested sugar-based solutions. For species detection, we allowed more than 400 mosquitoes to feed on one FTA card, but no species detection with a qPCR-based species identification assay was successful so far. FTA cards can be a useful complement for arbovirus surveillance, but their use was not effective to detect mosquito species.

Assessment of a mobile qPCR laboratory to test Guatemalan mosquitoes for DENV, CHIKV, and Zika viruses

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The overall goal of this study was to provide personnel and wholistic system training to assess the feasibility and practicality of a concise battery-powered laboratory to grow wild-collected larvae and pupae to adulthood, speciate and sex them with a microscope, and to perform mosquito grinding, RNA extraction, and qPCR arboviral testing. The specific technical goal was to attempt to detect evidence of vertical transmission of dengue, chikungunya and Zika within pools of wild-derived male and female adult mosquitoes. 635 adult mosquitoes were characterised, which were previously captured in the larval and pupal phase and then cultured for seven days, under controlled conditions of temperature, humidity, and feeding in the town of Zunilito Suchitepéquez, Guatemala. The samples come from Coatepeque, Quetzaltenango; Zunilito and Santo Domingo Suchitepequez. The samples comprised of the following: 267 females of *Aedes aegypti*; 328 males of *Ae. aegypti*; 25 females of *Anopheles albopictus*; 11 males of *An. albopictus*; and 4 *Culex*. The mosquitoes were maintained separately, based on collection location, and subsequently speciated and sexed via an expert using a common stereoscope. After sorting, mosquito grinding in VTM buffer, and total nucleic acid extraction was performed on each pool using a column-based commercial kit (CoWin Bio, China P.R.), to subsequently detect dengue, Zika, and chikungunya viruses by portable battery powered qPCR (CoDx Box, BMS, Australia) using VectorSmart™ kits (Co-diagnostics, Inc. USA). 3 qRT-PCR runs were performed, on 30 pools, with between 10 and 20 mosquitoes per pool. Endogenous internal extraction controls were included in every sample run, and were all positive, indicating good nucleic acid yield. 2 PCs and one NC were run per PCR experiment. Each control gave the expected results, indicating the PCR functioned well, despite being battery powered. Virus was not detected in samples, with no Cq values over 35. This study proves that qPCR testing for vector borne diseases can be successfully performed in the field using a portable laboratory and a battery powered qPCR. Extraction controls as well as positive controls were clearly evident in all samples tested making this a suitable technique for arboviral testing in mosquito samples in the field allowing for faster and more efficient deployment of control strategies in areas where mosquito carriers for Zika, dengue and/or chikungunya are present. Future work will focus on testing wild adult mosquitoes for the presence of various arboviruses using this portable system.

Strong electric field repel mosquitoes: initial field tests, using charged Venetian blinds

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Strong electric fields, generated between an array of differently charged conductors, prohibit flying mosquitoes from passing through. This repellent effect depends on the electric field strength (and thus the applied voltage and the distance between the conductors), the conductors' shapes, and their orientation towards each other. Previous laboratory studies had shown that electric fields of 2 kV/cm, produced between flat, parallel metal bars, could lower the number of mosquitoes passing through them in a given amount of time by more than 90%, compared to a non-charged set-up. One potential use of this technology is the use of charged Venetian blinds, installed in front of window openings. We first evaluated a variety of such Venetian blinds, all made by the company, Reflexa, in room tests and selected one design, which exhibited satisfactory repellence, even at 1 kV/cm. This specific design is also especially sturdy and robust, and the slats can be separated by up to 7cm. The blinds were then adapted to be electrically charged through a modified side rail system. The charge is supplied by a prototypical device ('Repuls Box'), which converts 12V into the necessary high voltage, while capping electrical currents to safe levels in the case the conductors are touched or are otherwise cut short. In the following field tests, the Venetian blinds were placed in front of a window of a camping tent, with a modified BG-Counter™ trap on the inside, with CO_2 as an attractant. Through its modification, it is not only possible to use the BG-Counter™ to remotely schedule the release of carbon dioxide and to automatically count the catches in real time, but also to switch the Repuls Box off and on from the distance. This set-up generally allows for a highly standardised and efficient test protocol and was installed at four locations. The trials showed a reduction of mosquitoes (*Aedes*, *Culex*, *Coquillettidia*) entering the camping tent by an average of more than 70% in the field. In our poster, we present data from different set-ups and discuss variables that influence the efficacy of the technology, as well as other potential use cases. This project is supported by the Federal Ministry for Economic Affairs and Climate Action (BMWK) on the basis of a decision by the German Bundestag.

Mark-Release-Recapture of *Aedes albopictus* in Portugal: the influence of climatic factors

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Aedes albopictus mosquitoes spread diseases like dengue, Zika, yellow fever, and chikungunya. The Sterile Insect Technique (SIT), which is based on production of male sterile mosquitoes through radiation and in the release in target areas, can be used as a prevention and control tool against *Ae. albopictus* native populations. Mark-Release-Recapture trials (MRR) estimate the number of mosquitoes to release during the SIT, but weather conditions can affect results. Thus, we aimed to understand the influence of climatic factors on mosquito collection. MRRs were conducted in October 2022 in Faro, Southern Portugal. Mosquito sterilisation, marking and transport followed protocols from the International Atomic Energy Agency. Releases occurred weekly for three consecutive weeks at two different sites, and mosquitoes were collected using Human Landing Collections (HLC) one, two, four and six days after release. Climatic data, such as temperature, humidity, wind intensity (moderate 15-35 km/h vs weak <15 km/h) and precipitation, were obtained from the Portuguese Institute of the Sea and the Atmosphere. We used generalised linear models with a negative binomial family and log function to estimate factors associated with the number of captured mosquitoes, estimated prevalence ratios (PRs), and the 95% confidence intervals (CI). We released 84 000 marked sterile male mosquitoes and recaptured 528 marked males (0.7%) by HLC. The prevalence of captured mosquitoes was 23% lower when the wind intensity was moderate compared with a weak wind intensity (PR: 0.77, 95% CI: 0.61–0.98). We did not find a statistically significant association between the number of captured mosquitoes and humidity (PR: 0.98, 95% CI: 0.96–1.00), temperature (PR: 1.03, 95% CI: 0.83–1.28) and precipitation (PR: 1.07, 95% CI: 0.60–2.03). Conclusion: It is crucial to plan MRRs carefully and consider weather conditions during their execution to avoid underestimating the population needed for the SIT and compromising the effectiveness of this control method.

New approaches to determine the age of *Culex pipiens* s.s. mosquitoes

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The age of mosquitoes is a critical determinant of their vectorial capacity. To become a vector, a mosquito should live longer than the extrinsic incubation period of the pathogen it carries, which may be quite long compared to mosquito lifespan in nature. Despite decades of research on age-grading methods, determining the age of wild mosquitoes still remain a challenging process. In this contribution, we present the results of two new approaches to calculate the age of *Culex pipiens* s.s. mosquitoes: the transcriptional age-grading technique, based on the differential expression of age-responsive genes; and an automated classification system trained with machine learning, based on differences in the flight pattern. While former has never been applied to *Culex pipiens* s.s., the latter is a totally new technique which has not been tested before in any mosquito specie. A cross-validated model was developed for each case reporting similar results: two separate groups of age (mosquitoes ≤4 days old and mosquitoes >4 days old) can be differentiated with acceptable levels of accuracy. These techniques are effective to discriminate young mosquitoes from older ones that may act as potential vectors, which is a matter of interest for research and surveillance programmes.

Improving spraying by air stream: spraying efficiently from 0.5 up to 13 m and saving up to 40% liquid

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The AS 1200 rechargeable spray blower: spraying in an air stream with pressure-controlled fluid supply (in combination with e.g. battery operated backpack sprayer REC 15). The patented AS 1200 (battery operated backpack sprayer with a wind machine) is now used worldwide by professional users in green industry, pest control, disinfection, construction etc. The stream of air generated by the blower is elongated and flows in a straight direction, so that the droplets are blown in the intended direction when they are released. This means that more of the solution lands on the plant itself compared to previous techniques. Spraying takes place in a defined manner: the pressure of the solution, the nozzle for spraying (various nozzles) and the airflow speed are optimally matched. This results in even distribution and foliage penetration or surface wetting. The controlled airflow significantly reduces drift. Less solution not only means lower costs – it also helps to protect the environment as fewer chemicals are used. The AS 1200 spraying system can be used for a variety of applications and achieves optimum results whether users need to spray a specific area with pin-point accuracy or treat flat areas dense foliage or hedges. The users questioned stressed the good penetration and wetting of the upper and lower sides of leaves. The adjustable spray pressure, the five levels for wind speed (plus turbo) and the choice of differently sized nozzles or special nozzles for mosquito control applications allow each user to adapt the device to their particular application. Testing the leaf deposition with tracer confirmed that the deposits with the AS 1200 are more or equal to the standard lance application. Users are on the lookout for products which save time, allow them to treat more effectively, produce no annoying exhaust gases and create less noise. The majority of users also consider saving solution or more effective spraying as a very important advantage. Depending on the application, savings of up to 40% can be achieved. Such savings are possible because there is less wastage. The users surveyed also mentioned that they save a great deal of time when working. Users reports time saving from 20% or even more depending on application with the AS 1200 than with conventional sprayers.

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