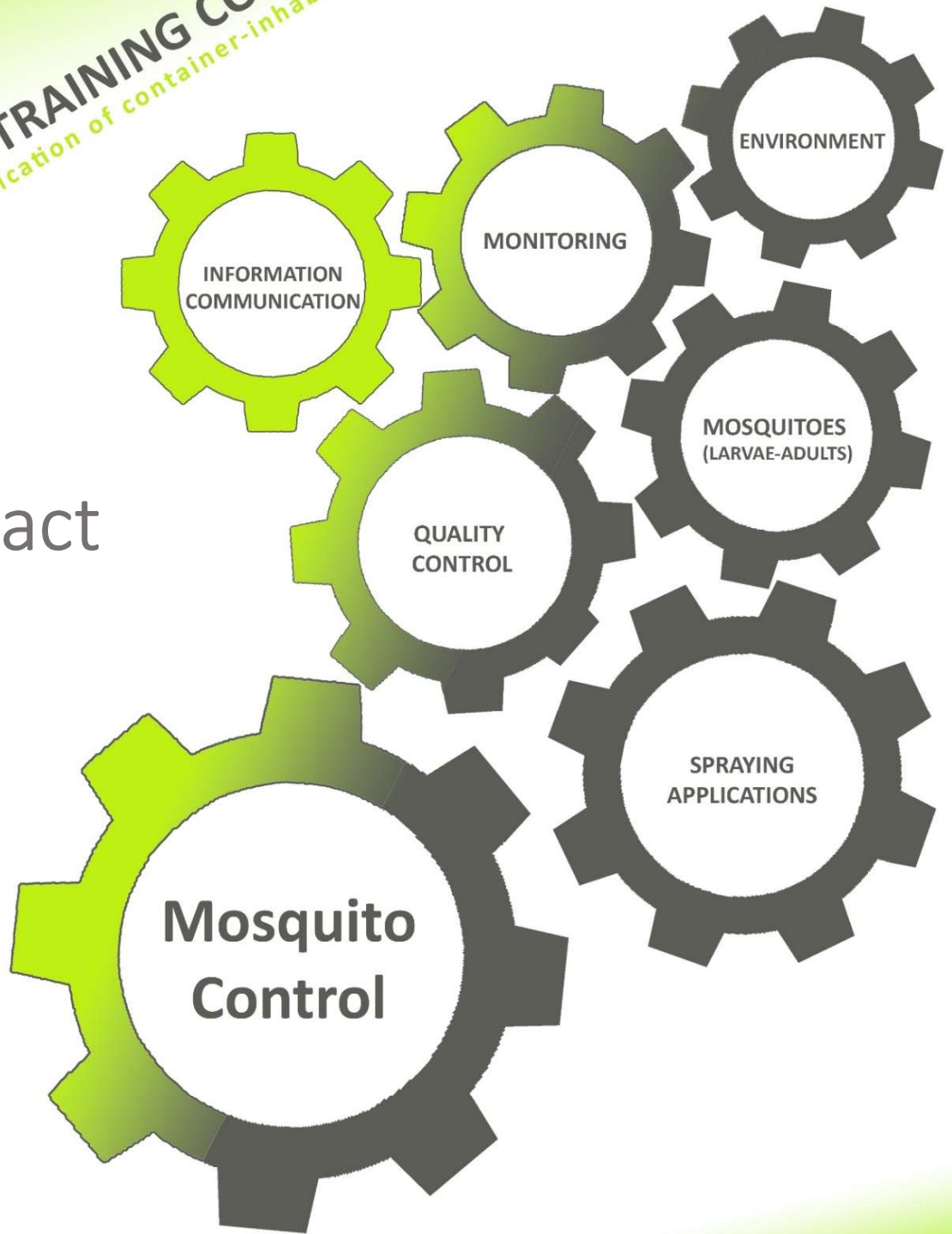


10th EMCA WORKSHOP 28-29/11/2022
Best Practices

3rd TRAINING COURSE
Identification of container-inhabiting *Aedes* mosquitoes' eggs

30/11/2022

Abstract
Book



The European Mosquito Control association would like to thank the following organizations for their contribution and support to the 10th EMCA Workshop:



With the support of the University of Applied Sciences of Southern Switzerland (SUPSI)

University of Applied Sciences and Arts
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Grégory L'Ambert	EMCA Member EID-MED, France

Training course

Eleonora Flacio	EMCA Board Member (Chair) Senior researcher, Vector Ecology Unit, Institute of Microbiology, University of applied sciences and arts of Southern Switzerland (SUPSI)
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Trainers:

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Welcome Address

In 2021, the EMCA released a brief questionnaire to identify needs and expectations from mosquito and other vectors surveillance and control community, but also stakeholders and public health officers, about our association's activities and services. It was open to both members and non-members. The highest ranked action was "Develop networking on best practices for vector control and surveillance". Therefore we did invite the mosquito control community to attend our 10th EMCA Workshop and to sit together and discuss "best practices for mosquito control in Europe" with the aim of producing guidance in a consensual process. In this interactive workshop, we address eight basic topics, for which best practices need to be determined:

1. Best practices for surveying adult mosquitoes to define the target
2. Best practices for surveying mosquito larvae for decision-making upon larviciding
3. Best practices for mosquito control in wetlands
4. Best practices for mosquito control in the rural/periurban system
5. Best practices for mosquito control in the urban system (public areas)
6. Best practices for mosquito control in the urban system (private areas and points of entry)
7. Best practices for the comparison of available monitoring and control tools
8. Best practices for efficacy evaluation in mosquito control

The workshop audience is invited to contribute through working groups and plenary discussions, and guidance documents will be finalised by the 'best practices' (BP) EMCA Working Group before dissemination. On behalf of the EMCA I'm very glad to thank all BP team members for their investment in the project, but also all workshop participants for, without a doubt, their valuable contribution. We all thank SUPSI and in particular Eleonora Flacio and her team, for kindly hosting the event in Mendrisio. Acknowledgments are also deserved by our sponsors, who allow us to welcome participants with reduced registrations fees.

Our workshop will be followed by the 3rd EMCA Training Course, on the "Identification of container-inhabiting *Aedes* mosquito eggs", also kindly hosted by SUPSI.

Describing 'best practices' for mosquito control is challenging but essential to promote the use of the most efficient and less damageable methods and to get mosquito control accepted by the community. I wish to all participants to enjoy the workshop and the training course, and I look very much forward to the outcomes!

Francis Schaffner
President of the EMCA

Full Programme

Monday 28 th November 2022		
8:00-9:00	Workshop Registration	
9:00-9:05	Opening address and welcome by the director of the Institute of Microbiology, SUPSI	Prof. Mauro Tonolla
9:05-9:15	What means 'best practices' and why do we need them?	Francis Schaffner
9:15-9:45	<u>Keynote lecture</u> : The needs and challenges in vector surveillance and control in Europe	Olivier Briët
9:45-10:05	Update about the organisation of mosquito control in Europe	Sandra Gewehr
10:05-10:25	State of the art of the "One health approach" in practice	Sophie Dhollander
10:25-10:45	Environmental impact assessment of mosquito control in wetlands and floodplains. Survey and sampling methods of non-target organisms and inferences on prey availability in complex ecosystems.	Thomas Weitzel
10:45-11:10	Coffee/Tea break	
11:10-11:30	State of the art of predictive modelling for mosquitoes and mosquito-borne diseases for use in mosquito control	William Wint
11:30-11:50	The use of operational modelling in wide-area mosquito control	Spiros Mourelatos
11:50-12:10	Insecticides and new paradigms in mosquito control: current status, problems and perspectives	John Vontas
12:10-12:40	<u>Keynote lecture</u> : Best practices and requirements for developing normative documents on vector control according to WHO norms and standards	Florence Fouque
12:40-13:30	1 st Poster session	
13:30-14:30	Lunch	
14:30-15:30 15:35-16:30	8 Working Groups (WG) Session A Session B	WG coordinators & Facilitators
16:30-16:50	Coffee/Tea break	
16:50-18:00	2 nd Poster session	
18:00-19:00	EMCA Annual General Meeting	
20:00-open end	Dinner	

Tuesday 29 th November 2022		
9:00-9:15	Best practices for surveying adult mosquitoes to define the target – WG report	Francis Schaffner
9:15-9:30	Best practices for surveying mosquito larvae for decision making upon larviciding – WG report	Sandra Gewehr
9:30-9:45	Best practices for mosquito control in wetlands – WG report	Grégory L'Ambert
9:45-10:00	Best practices for mosquito control in the rural/periurban system – WG report	Ruben Bueno-Marí
10:00-10:15	Best practices for mosquito control in the urban system (public areas) – WG report	Spiros Mourelatos
10:15-10:30	Best practices for mosquito control in the urban system (private areas and points of entry) – WG report	Adolfo Ibáñez-Justicia
10:30-11:00	Coffee break	
11:00-11:15	Best practices for the comparison of available monitoring and control tools – WG report	Andreas Rose
11:15-11:30	Best practices for efficacy evaluation in mosquito control – WG report	Andrea Drago
11:30-13:00	Plenary discussion on Best Practices for Mosquito Control in Europe	
13:00-13:30	Recapitulation – What are the next steps? Which other best practices should be addressed in the future? Closure of the workshop	Francis Schaffner & Facilitators
13:30-14:30	Lunch	
Wednesday 30 th November 2022		
8:00-18:00	Training course “Identification of invasive <i>Aedes</i> species eggs” (<i>Aedes aegypti</i> , <i>Ae. albopictus</i> , <i>Ae. japonicus</i> , and <i>Ae. koreicus</i>).	Eleonora Flacio & collaborators

Invited Speakers' Abstracts

Oral presentation 1

What means ‘best practices’ and why do we need them?

Francis Schaffner

President of the EMCA

Francis Schaffner Consultancy, Riehen, Switzerland

‘Best practices’ refer to a set of methods of choices in terms of efficacy, cost effectiveness, ecological soundness and sustainability. Portraying these practices may help to promote their application while avoiding the use of less efficient and more damageable methods (to public health and to the environment) and to get mosquito control better accepted by the community. The best practices will ensure the mosquito control actions to be adapted to the local context in terms of target species, local environment, and objectives. A panel of control methods exists to implement the best practices and the source reduction (avoiding mosquito breeding) must always be prioritised to avoid the use of inputs in the environment. Best practices may be based on Integrated Vector Management (IVM) strategy, which combines a number of complementary methods within a “rational decision-making process for the optimal use of resources for vector control”.

Oral presentation 2

The needs and challenges in vector surveillance and control in Europe

Olivier Briët

European Centre for Disease Prevention and Control (ECDC)

The objective of this keynote address is to describe the needs and challenges in vector surveillance and control in Europe.

A survey was conducted among selected European medical and veterinary entomologists. This survey shows that European medical and veterinary entomologists most often identify lack of financial resources, and related, lack of human resources, as challenges to vector surveillance and control. Also, the arsenal of registered biocides is considered to be limited. Further, there is a need for evidence-supported guidance on principles and targets of control: when to deploy what and to what effect.

Evidence on cost-effectiveness of vector control strategies/tools is scarce. Cluster randomized controlled trials (CRCTs) with arboviral disease cases as outcomes are impractical/expensive in non-endemic and epidemic settings such as in Europe, but CRCTs with entomological outcomes are more feasible and affordable. A recent literature review commissioned by EFSA in the VectorNet project shows that entomological outcomes correlate with epidemiological outcomes in terms of intervention effect. With help of modelling, vector control impact on disease epidemiology can be predicted from results of entomological CRCTs.

In order to respond to the expressed needs of more guidance on vector control, and of more public resources for vector control (which could stimulate development and registration of biocides), more studies that demonstrate cost-effectiveness of vector control strategies are needed to inform guidance and to advocate for more public resources for vector control.

Oral presentation 3

Update about the organization of Mosquito Control throughout Europe.

Sandra Gewehr

Ecocodevelopment S.A, 57010 Filyro, Thessaloniki, Greece

Since the recent emergence and re-emergence of mosquito-borne diseases in Europe, several research has been conducted to identify gaps and needs for mosquito surveillance and control in Europe mainly through questionnaires to involved stakeholders with the ultimate goal of harmonising procedures and developing a common strategy.

Guidelines for the surveillance and control of mosquitoes of public health importance in Europe are practically issued only the last ten years, by four major international bodies: WHO, ECDC, EFSA and EMCA. These guidelines are calling for evidence-based decision making, use of a broad range of control strategies according to the Integrated Vector Management (IVM) approach and strengthening of community involvement, pointing out the need for harmonisation of methods applied.

The question is, out of the plentitude of methods, tools and strategies suggested by these guidelines and scientific advice, which are actually being adopted and regulated within national health policies, and which institutions are in charge of key functions.

To this goal, in the frame of a questionnaire conducted by the AIM Cost network (Aedes Invasive Mosquitoes Cost Action, Wint et al. 2020) in 2019, 154 respondents from 36 countries throughout Europe provided 66 documents, stakeholders were using in their countries for the surveillance and control of Aedes Invasive Mosquitoes. Through an additional questionnaire which addressed also native mosquitoes and was conducted by the author, 41 additional documents were gathered from 32 stakeholders of 19 countries. The total of 107 documents includes international guidelines, scientific articles and a plethora of national and regional policy papers including legally binding documents (law, Public Health Act, decrees, ordinances, decisions and circulars), technical advice-giving documents (guidelines, action plans, handbooks, protocols, management plans and a training curriculum) as well as position papers and technical reports.

In this communication preliminary results from 41 policy papers from 13 countries are presented regarding the topics regulated and the responsibilities of stakeholders involved in Mosquito surveillance and control in Europe. On the basis of examples from four countries, strengths and weaknesses of different types of organisation of mosquito control are elaborated, resulting in some first suggestions concerning the interplay of different stakeholders involved and political priorities that will be essential for an effective response to the increasing risk of mosquito-borne diseases in Europe.

Oral presentation 4

State of the art of the One Health approach for the prevention and control of vector borne diseases.

Sophie Dhollander

European Food Safety Authority, Parma, Italy

Vector-borne pathogens have the potential to spread rapidly in previously unaffected areas, possibly causing severe impact on public and/or animal health, resulting in important economic and/or environmental consequences. Early detection of new incursions of VBD-pathogens is therefore of primordial importance and a set of prevention and control options should be readily available for the most harmful pathogens to avoid their spread. Efforts to implement a one-health approach for vector prevention and control are challenging, due to the complexity of the epidemiology of vector borne pathogens and lack of good quality data on the effectiveness of the different control options. Efficient control options are rarely available or not economically interesting. The resulting compromises are not always meaningful for all partners involved. For instance, several different European institutions are involved in safety assessments and approval of biocidal products used for vector control, assessing, in parallel, either their impact on the environment, food and feed safety, public health, or animal health. Although some biocidal products are effective at reducing vector-borne disease transmission rates, their widespread and long-term usage has environmental implications. On the other hand, the specific use of some biocidal substances may be approved but might not be applicable or efficient to control the vector species involved in the transmission of the newly emerging pathogen. To complicate the picture, the lack of clinical signs in reservoir hosts infected with some vector-borne pathogens represents a lack of incentive to implement vector control by veterinary services or wildlife protection bodies. A true one-health approach for prevention and control of vector borne diseases will be to develop joint decision trees based on holistic risk assessments and a profound knowledge of the disease epidemiology. Whilst they should involve all potential stakeholders in the decision making by creating one health bodies, surveillance efforts for early detection should focus on those epidemiological compartments with the highest probability of detection of the pathogen. Control efforts should focus only on those measures with the high probability of reducing the overall impact of the disease, also when this represents an economic burden for those that are not immediately affected by the spread of the pathogen.

Oral presentation 5

Environmental impact assessment of mosquito control in wetlands and floodplains - Survey and sampling methods of non-target organisms and inferences on prey availability in complex ecosystems.

Thomas Weitzel

Kommunale Aktionsgemeinschaft zur Bekämpfung der Schnakenplage e.V. (KABS), Germany, Georg-Peter-Süß-Str 3, D-67346 Speyer

The environmental impact of mosquito control in wetlands and floodplains is discussed concerning the reduction of target species, the potential impact of agents on non-target insects and food-web disturbance. For all those interested, knowledge about sampling techniques, data quality, the biology of taxa and the relative importance of environmental factors is useful to see through various studies.

Commonly, targeted use of Bti (*Bacillus thuringiensis israelensis*) at the right time with adequate dosage is considered the least interfering control method with limited risk to other Nematocera. Therefore, particularly Chironomidae populations were studied by varying means and at different scales in variable biotopes combined with different intensity of Bti-application.

Chironomid production of treated and untreated areas can be compared as well as the situation before and after treatments. Larvae can be sampled through repeated substrate removal and adults by emergence traps. More unspecific, adults can be collected by light traps, sweep nets, suction traps, sticky cards and yellow pan traps.

The quality of data depends on the trapping protocol, the methods and the time-frame in relation to the size and complexity of the biotope and to the objective of the study. Ideally, the species abundance and diversity, the temporal dynamics and the biomass is quantified. Thereof, population trends in relation to application intervals and also to varying environmental factors can be detected. Despite counting individuals and species, productivity of a biotope should be quantified by biomass to estimate food web contribution.

Suspected food-web effects on aquatic, terrestrial and aerial predators of target and non-target species were studied. Mobility of predators and prey complicates the analyses, together with numerous environmental variables. Different quality and size of habitats and the dynamic complexity of food webs hamper predictions and definite conclusions. Therefore, adequately scaled long-term studies may detect trends beyond temporal fluctuations.

Some studies analyse diet composition of predators, others try to quantify an indirect impact of mosquito control on predator abundance or reproductive success. Consumption of Nematocera by Odonata has been shown by immunoassay. Seasonal Odonata occurrence can be inspected visually. Biweekly collections of exuviae along the banks of waters represent autochthonous breeding site productivity. Diving beetles, important mosquito larvae

predators, can be surveyed visually, by nets and by bottle traps. Nematocera, still less mosquitoes, made up little proportion of food of frogs, formerly analysed through stomach flushing.

Mosquitoes were found rarely in the diet of tetrapod aerial feeders, but Chironomidae represent a major food of some bat species and house martins, when they hunt over water surfaces. As always for bats, faecal analyses are standard for birds, since neck-collar method is obsolete today. Faeces bring along some constraints concerning quantification of poor remains of small prey and taxa determination, which usually ends up at order and sometimes at family level.

Oral presentation 6

State of the art of predictive modelling for mosquitoes and mosquito-borne diseases for use in mosquito control

William Wint (remote talk)

Environmental Research Group, Oxford

Mosquito control may be aimed at disease or nuisance mitigation. Modelling to assist with control operations most frequently focusses on identifying and locating current and/or future target insect populations.

Predictive modelling for mosquitoes comes in many forms. Two widely used types are mathematical modelling of vector populations and spatial modelling of vector distributions. Both these methodologies can be designed to fill gaps in existing knowledge about a vector's current numbers and distributions, and to predict these parameters into the future. Most methods require calibration using known ("training") data from field sample or lab experiment

There are a number of important factors that need to be considered when building these models including geographical extent, consistency of training data, use of relevant standardised covariate datasets, parameters to be modelled, and of course, the appropriate choice of modelling methodology. Equally important is the definition of outputs: - level of detail, matching outputs to user requirements, producing clear maps and tables, clear reporting, and dissemination to downstream users.

This presentation provides an overview of some of the main issues to be considered when implementing mosquito models for control.

Oral presentation 7

The use of operational modelling in wide area mosquito control

Spiros Mourelatos

Ecodevelopment S.A., 57010, Filyro, Greece

The easy access to the plethora of remote sensing data and the wide spread of AI methodologies has led to a lot of models on vector borne diseases worldwide. Nevertheless, the majority of those models are developed at coarse spatial and temporal scales (e.g. county/annual) and are not really useful for many of the decisions needed for mosquito control operations. Hence, out of the 41 West Nile Virus predictive models developed in the USA, only three are potentially capable to guide mosquito control actions (Keyel et al, 2021).

The operational modeling is a valuable compound of early warning systems in vector borne diseases such as the EYWA (Early Warning system for mosquito borne diseases), a system awarded with the 1st EIC (European Innovation Council) Prize in January 2022. EYWA is a consortium of fifteen partners from five countries, out of which seven are active members of the EMCA. Within EYWA, we are sharing entomological and other field data produced seamlessly already from 2010 and onwards. This endeavor started in 2019 and will be pursued until 2025, aiming at securing the optimization of its digital products and their translation into decisions and mitigation actions in wide area mosquito control. EYWA has three core members (National Observatory of Athens-NOA- coordinator, University of Patras-UPAT, Ecodevelopment-ECODEV) which have delivered until today seven products: four entomological and WNV risk predictive models (MAMOTH- NOA, MIMESIS- UPAT, BAd and BAR- ECODEV), an application (Mosquito Vision- ECODEV), a spatial pattern model (Urban Vision- ECODEV) and the EYWA platform (NOA).

In this presentation, I will briefly show three operational models of Ecodevelopment:

- BoL, a weekly predictive model for the presence-absence of mosquito larvae on a breeding site-specific scale (operational in the Region of Central Macedonia since May 2022 and Western Greece since September 2022)
- BAd, a daily-run predictive model for the abundances of adult *Culex spp.*, *Aedes spp.* and *Anopheles spp.* mosquitoes on a settlement level (operational in 2,415 settlements in four regions of Greece since 2020).
- BAR, a weekly predictive risk model for West Nile Virus (WNV) on a settlement level (operational in Central Macedonia from 2020 for 1,000 settlements)

and the way these products are supporting our operations in terms of critical decisions in the course of wide area mosquito control in 40% of Greece: mosquito trapping, WNV screening in mosquitoes, WNV screening in Sentinels, larviciding intensification, adulticiding (ULV, LV), micro stratification in the urban, public outreach, resource allocation, project strategy.

The members of the EMCA that are performing wide area mosquito control throughout Europe, have to deal with critical decisions such as the ones above mentioned and by using systems such as the EYWA will be facilitated in their mission.

Keyel AC, Gorris ME, Rochlin I, Uelmen JA, Chaves LF, Hamer GL, et al. (2021) A proposed framework for the development and qualitative evaluation of West Nile virus models and their application to local public health decision-making. *PLoS Negl Trop Dis* 15(9): e0009653. <https://doi.org/10.1371/journal.pntd.0009653>

Oral presentation 8

Insecticides and New Paradigms in mosquito control: current status, problems and perspectives

John Vontas^{1,2} (remote talk)

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Mosquitoes and vector borne diseases have re-appeared sporadically in some regions in Europe due to several factors, such as climate changes and anthropogenic reasons. Environmental management and use of insecticides are effective means to control mosquitoes. Several well-documented success stories indicate that insecticide can reduce mosquito populations and diseases, but chemical control may not be sufficient, due to the development of insecticide resistance in most mosquito vectors and the limited number of environmentally safe insecticides. Several alternative novel vector control tools (VCTs) have been developed in the last decade, including Attractive Toxic Sugar Baits, Auto-dissemination, Mass trapping, Spatial Repellents, Wolbachia, modern SIT and transgenic technologies. The adaptation of these alternative New Paradigms depends on their strengths and weaknesses, regulatory considerations, and operational constraints. Conditions where these alternative vector control methods/tools could offer greatest public health value will be discussed.

Oral presentation 9

Best practices and requirements for developing normative documents on vector control according to WHO norms and standards

Florence Fouque

Research for Implementation Unit, The Special Programme for Research and Training in Tropical Diseases, World Health Organization, 20, avenue Appia, CH-1211 Geneva 27, Switzerland

The process within the World health Organization (WHO) for developing normative documents on vector control includes two main streams starting with the recognition of the efficiency, benefits and no-harms of the intervention (products and tools) and followed by the development of guidelines. Both streams are under different WHO working departments with the first one under the operational departments and the second one into the Science Division department, to guarantee the transparency and independent decisions on recommendations and guidelines. The first part of the review will be looking at technical elements when the second part will be more looking at respect of norms and standards. The first part of the process has been recently revised for clear definition of the norms, standards and processes underpinning the development of WHO recommendations on vector control. It starts with the pre-qualification for different pathways for interventions with an existing WHO recommendation and interventions with no recommendation. Then the review of the interventions for efficacy and no-harms are moving to the qualification pathways with different evaluation. The efficacy of a vector control intervention is scrutinized for both entomological and epidemiological impact. The outcome of the qualification evaluation is then published. For new interventions proposed by the developers, this review is made by the WHO Vector Control Advisory Group (VCAG) constituted by external and independent experts. When the outcome of the evaluation provides positive feedback to move the intervention to recommendations and guidelines, then the WHO operational department is responsible for constituting the different review groups that will work on the final recommendations and guidelines. A first planning proposal is then submitted to the WHO Guidelines Review Committee (GRC) and WHO experts from the GRC will review the proposal not on technical elements but on the respect of the Quality Norms and Standards (QNS). This review provides an approve, approve with revision, or resubmit response. Once the planning proposal approved, the development of the guidelines can start, and the final guidelines are again submitted to the GRC review. For already recommended vector control interventions the process for reviewing the existing recommendation can take about one year if evidence is available and convincing. But for new intervention the full process for new vector control intervention can take from two years and more to move from VCAG review to final guidelines published.

Poster Presentations Abstracts

Permanent monitoring of *Aedes albopictus* in Emilia-Romagna region, Italy

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² Emilia-Romagna region Public Health Department, Bologna, Italy

Emilia-Romagna region, Italy, conducts standardised *Aedes albopictus* monitoring by ovitraps during summer seasons in all nine provinces of the region since 2008. Starting from 2017 the number of monitoring ovitraps was reduced from about 2650 (involving about 240 municipalities) to 755 positioned in the main cities only. Before being added to the database, data are submitted to a validation process through a specific protocol. Every month (June, July, August, September) density maps of the species are produced from ovitraps data interpolation (Inverse Distance Weighted) for supporting mosquito control activities in the cities. All the monitoring data are published and freely available in the web site www.zanzaratigreonline.it. In this work we report the summary data and the density maps of the last five years (2017-2021) compared to updated ovitraps data 2022.

Integrated control of *Aedes albopictus* in allotment gardens, residential areas and sewerage systems in Franconia, Germany

Judith Auer¹, Silke Göttler², Nico Jacob¹, Alexander Kassel¹

¹APC AG; Nürnberg, Germany

²Biogents AG, Regensburg, Germany

The mosquito species *Aedes albopictus* has been established as an invasive species in Germany over the last 15 years. Currently, at least 25 overwintering populations have been detected, mainly in southwestern Germany. In 2019, individual specimens were found for the first time in allotments and residential areas in Fürth, Germany, and were confirmed as a wintering and established population in 2020. Due to the expansion of the population, control measures were commissioned from September 2020 until the end of 2023. The control concept includes 1. mapping of the area and documentation of all breeding sites; 2. information of citizens with a call for active cooperation; 3. control measures in all gardens and public sewers with *Bacillus thuringiensis israelensis* (Bti) and use of 600 traps (artificial breeding sites; BG-GATs); 4. identification of hotspots and quality control. An independent monitoring programme with different kinds of traps controlled the measures and investigated the further spread of *Ae. albopictus*. The tiger mosquito population in Fürth was severely depleted in 2021 compared to the previous year, as shown by monitoring data from Biogents (67% decline in the entire control area) and APC (86% in an allotment area). Monitoring data for 2022 are currently being collected, with preliminary data showing similar numbers of *Ae. albopictus* compared to 2021. Reasons for this include the extremely hot and dry summer of 2022, which resulted in a shortened mosquito development period and increased use of artificial breeding sites (BG-GATs). Due to low rainfall, water in the sewer system stood over the summer and had to be treated with a much higher concentration of Bti due to contamination by organic material. In order to sustainably control the tiger mosquito population in Fürth, monitoring and control measures must continue in subsequent years.

Entomological surveillance of mosquitoes (Culicidae) in the Region of Attica as part of an integrated mosquito management

Georgios Balatsos¹ and Antonios Michaelakis¹

¹ Scientific Directorate of Entomology and Agricultural Zoology, Benaki Phytopathological Institute, 14561 Kifissia, Greece

Entomological surveillance is essential for both operational and research purposes. Within the framework of the systematic entomological surveillance of the mosquitoes in the Region of Attica, a total of 55 BG-Sentinel 2 (BGS2) traps and 110 oviposition traps (ovitrap), were installed. For the selection of the traps' locations, the geostatic method of stratified random sampling was used, based on land use criteria. The BGS2 traps equipped with BG-Lure and constant flow rate of CO₂ (gas cylinders), were continually operated. Traps (BGS2 and ovitrap) were inspected weekly throughout the year. Eighteen (18) mosquito species have been recorded, among which the species *Culex pipiens* and *Aedes albopictus* were the most abundant. In 2021, the highest number of *Cx. pipiens* adults were captured from the 1st week of June to 1st week of July and in 2022, from the 2nd half of June to 2nd week of July, whereas for *Ae. albopictus*, in 2021, the highest number of adults were captured from 1st to 4th week of July while in 2022, from 2nd week of September to end of September (both for adults and eggs). Captured *Culex pipiens* adults were further analyzed to determine the infection rates of the West Nile virus (WNV). Based on the results, interactive and static thematic maps for *Ae. albopictus*, *Cx. pipiens* and *Anopheles* sp. are being produced and communicated weekly to the relevant stakeholders (both at Regional and local level) to support integrated mosquito management. Especially for invasive mosquito species, the results from the entomological surveillance will be further evaluated to develop innovative tools for their management, such as the Sterile Insect Technique (SIT), "hot spot" approach, etc.

Keywords: Attica, Mosquitoes, Regional Unit, Entomological surveillance, Stratified random sampling.

Within the framework of the project «moSquTo»: Innovative approaches for monitoring and management of the Asian tiger mosquito with emphasis on the Sterile Insect Technique (TAEΔK06173- National Recovery and Resilience Plan, "Greece 2.0" & EU Funding – Next Generation EU) and the "Research project for the entomological surveillance of mosquitoes in Attica Region (Athens, Greece)".

Taking a “rain check” from field work in Sweden

Christian Blue¹, Martina L. Schäfer¹, Jan O. Lundström^{1,2}

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Biologisk Myggkontroll has developed a reliable method for accurate remote definition of floodwater mosquito larvae occurrence at any given time. However, the method still required repeated visits to many distant field sites after rain to see if water level has increased and if the larvae are present. In the absence of hydrological data, a disproportionate amount of fieldwork can go into monitoring the need for mosquito control. This has been the case in Sweden with smaller and often more remote floodplains. With the goal of minimising false flags and thereby preventing unnecessary fieldwork, we began installing hydrostatic probes to gauge changes in water depth. After a successful pilot, we refined the methodology and expanded the programme. This poster seeks to provide a baseline best practice for remote monitoring of floodplains through hydrostatic probes based on our experience but also to encourage discussion among experts in the field (YOU) as to how we could refine the programme further.

Knowledge, Attitude and Practices towards the Asian tiger mosquito among primary school children in Torrent, Spain

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Aedes albopictus (Skuse, 1894) was first detected in the province of Valencia (eastern Spain) in 2013. Since then, this invasive mosquito has become a serious pest due to its annoyance and its importance as a public health concern. Within the framework of the research project "New strategies for the control of the tiger mosquito in residential areas", also known as NESCOTIGER, several awareness activities were carried out during 2021 and 2022 in the Valencian municipality of Torrent. One of these activities was the realization of awareness talks on basic aspects of the biology, prevention, and control of *Ae. albopictus* to primary school students. A questionnaire based on those previous topics was voluntarily filled by participants before attending. Data were obtained by a structured anonymous questionnaire (20 questions) that was filled out by 546 students belonging to six different primary schools located in the residential area of El Vedat (ages 8-13, mean=10.3 years old) during March and May 2022. Of all students, 70.1% (n=382) knew about the presence of *Ae. albopictus* in Torrent, and 84.6% (n=461) confirmed that their families carried out at least one control or prophylactic measure against the Asian tiger mosquito in their households. In addition, 35.4% (n=193) were aware of the role of this mosquito as a disease vector. Also, when asked about potential breeding sites in a multiple-answer question, most students answered that this mosquito species can breed in either piles of garbage or in the ground (49.5% and 49.9%, respectively), whilst the least responded breeding places were animal drinking troughs and empty bottles or cans (11.9% and 8.1%, respectively). Our study found a medium level of knowledge and preventive practices, which highlights the need for extensive educational and prevention programs related to this vector in primary school children of El Vedat.

Quality assessment of mosquito mass trapping implementation and its intrinsic efficacy in a field study for *Aedes albopictus* control

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Aedes albopictus is sustainably spreading across Europe and becoming a growing threat for public health. Exploitation of its oviposition behavior through traps represent an opportunity with limited environmental and health impact. The NESOTIGER project executed in El Vedat de Torrent (Spain) aimed to assess the efficacy of a mass trapping approach in a large-scale field trial. Inhabitants of this residential area were involved in this community-based intervention and provided with adulticidal oviposition traps (AOT) and larvicidal oviposition traps (LOT) targeting adult gravid female and immature stages respectively. Nearly 1500 AOT and LOT were deployed in the study area by residents, who were informed about the proper trap location in their gardens and its maintenance. Two inspections were conducted in random houses during summer 2022 for assessing these aspects in a null precipitation time (June) and after late summer rains (September). Mosquito breeding positivity and adult emergence from water contained in the positive traps were also evaluated. From the 75 inspected traps in June, 81.3% were properly maintained (contained water) and 45.3% were correctly installed, being both requirements met by 41.3% of the traps. Figures recorded in September for 105 units were lower in water filling (57.1%), proper location (37.1%), and considering both variables (32.4%). No larvae nor pupae positive AOT was found in June among the 31 that contained water, and one of the 35 traps of September was positive, and lead to the emergence of a single *Ae. albopictus*. Positivity of LOT was 30.0% (12/40) and 36.0% (9/25) in June and September's inspections respectively. Two *Ae. albopictus* adults emerged from two of the positive water samples in LOT inspected in June and September. Quality in resident's implementation of the trapping intervention appeared to be limited while both traps showed good intrinsic efficacy.

Multiple detections of *Aedes albopictus* in Belgium through Citizen Science.

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Aedes albopictus is an important vector of several pathogens of human and animal health importance. Currently, it is spreading northward in Europe, and Belgium is at the front of its invasive range. Recently, a passive mosquito surveillance through a citizen science platform was added to the existing active surveillance at parking lots. After the start of the passive surveillance in Belgium (www.muggensurveillance.be), *Ae. albopictus* was detected for the first time outside known points of entry (PoEs).

Since the launch of the citizen science website at the end of May 2022, *Aedes albopictus* has been reported at seven locations through pictures uploaded on the platform i.e., at Boorseme (BO), Kallo (AB), Lebbeke (LE), Wondelgem (WO), Antwerp (AN), Wilrijk (WI) and Grimbergen (GR). Only AB (a used tyre import company) is a well-known PoE, where all life-stages of *Ae. albopictus* were found in 2018. Between June and August 2022, AB, AN, BO and LE were actively surveyed, by larval sampling (two door-to-door visits per site) and oviposition traps in a 200 m buffer zone (placed for two weeks). Sites GR, WI and WO will be surveyed in the near future.

At AN and BO the surveillance did not yield new individuals of *Ae. albopictus*, whereas at AB new female mosquitoes and some eggs were collected. Despite the preventive larviciding applied at AB in May–July 2022, *Ae. albopictus* was detected in August 2022, but adults may have been introduced by newly arriving containers. An extra larvicide treatment was applied in the same month. At LE all life stages were collected and biting nuisance was reported by the inhabitants and the surveillance team. Therefore an intensified monitoring campaign was set-up from August until October 2022. The responsible authority (Flemish Region) was informed and control actions will take place at this location in September 2022.

It is clear from these findings that *Ae. albopictus* enters Belgium at many places in 2022, with even summer reproduction in some of them. Obviously, the passive surveillance by citizens provides an added value to the active surveillance by detecting *Ae. albopictus* also outside known PoEs. It will be important to follow-up these locations in 2023 to investigate possible overwintering, which would indicate the establishment of *Ae. albopictus* populations in

Belgium. Also the exact import pathways of *Ae. albopictus* at these localities are not known and warrant further investigation.

Establishment and operation of the Swiss Mosquito Network for surveillance and control of invasive mosquitoes

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Since their appearance in Europe, invasive *Aedes* mosquito species have represented a threat to public health and there is need for surveillance and control. Besides Canton Ticino, where invasive mosquito surveillance has been ongoing since 2000, several Cantons have now started to face the problem. Following the publication of the guidelines for the surveillance and control of invasive *Aedes* mosquitoes in Switzerland in 2016, between 2017 and 2019 we built up the Swiss Mosquito Network (SMN), which is operational since 2020. Its aim is to coordinate the surveillance and control activities across Switzerland and link them with the neighbouring countries, as mosquitoes are a cross-border issue. The network is founded by the Federal Office for the Environment (FOEN/BAFU) and consists in a coordinating office (SUPSI), the national surveillance programme (Swiss TPH), four regional reporting units (SUPSI, Swiss TPH, City of Zurich, University of Lausanne) and the Swiss Centre for Cartography of the Fauna (SZKF/CSCF). The SMN provides support to the Cantons in setting up a surveillance programme and advises them on control measures. Further, it collects reports of suspected invasive mosquito species from the citizens through a dedicated web page and carries out inspections in case of Asian tiger mosquito findings at new locations. This approach allows to collect all the data on Asian tiger mosquito findings centrally at national level and to forward the data to the info fauna – CSCF, the Swiss Centre for Cartography of the Fauna.

Field efficacy of VectoMax® FG biological larvicide against *Aedes albopictus* and *Culex pipiens* in urban catch basins

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The exotic invasive tiger mosquito, *Aedes albopictus*, appeared in southern Switzerland in 2003. The spread of the mosquito has been surveyed constantly since then, and an integrated vector management has been implemented to control its numbers. In public areas, the control measures focus on the aquatic phase of the mosquito with removal of breeding sites and applications of larvicides, mainly in catch basins. VectoMax® FG (Valent Biosciences) is a combined larvicidal formulation of *Bacillus thuringiensis* var. *israelensis* (strain AM65-52) and *Bacillus sphaericus* (strain ABTS-1743). This biological mosquito larvicide presents the advantage first to be specific to mosquito larvae, also of having a longer efficacy, compared to other similar products, reducing therefore the application efforts both in terms of quantity of product used and personnel employed. In 2021 we tested the effectiveness of the product VectoMax® FG against *Aedes albopictus* and *Culex pipiens* under real conditions in urban catch basins in southern Switzerland with the aim of giving the realistic guidelines to the local authorities in charge of the application.

Experience from the use of a web-based platform for the preparedness for and response to West Nile Virus outbreaks in Greece

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The repeated outbreaks of West Nile Virus (WNV) in Greece during the last thirteen years induced a shift in the strategy of large-scale mosquito control projects from the protection against nuisance towards the protection of public health. This shift concerns the targeted mosquito species as well as the environmental system focused on and the control methods to be implemented in different scenarios.

The EMPROS platform is the result of a co-design from stakeholders in research, public health administration and operators involved in the field of mosquito-borne diseases and mosquito control which aggregates, harmonises and visualises historical and actual monitoring field data concerning entomological surveillance and pathogen circulation as collected within the monitoring networks of Ecodevelopment SA. Field data are enriched with environmental proxies such as NDVI, NDMI, NDWI and NDBI from open-source Earth Observation data, geomorphological and meteorological data for the sampling date and with weather predictions for the upcoming 10 days.

This complex dataset as a whole is used for the development and further amelioration of a full suite of both, deterministic and data-driven predictive models, for the abundance of *Culex pipiens* and for WNV risk on a municipality down to a settlement level and from monthly down to weekly predictions.

The platform implements a role-based access to provide per-region data to specific users from the public health authorities and operators and first reactions indicate that the EMPROS platform represents a practical tool which provides supportive services for decision-making in the frame of wide-area integrated vector control projects aiming at the control of WNV outbreaks in Central Macedonia to the relevant stakeholders.

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Heat and aridity as antagonists of mosquitoes in Austria in 2022

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Usually, warm weather conditions promote mosquito population's development and hence number of mosquitoes due to higher reproduction rates, but in combination with striking lack of precipitation it turns into the opposite. Water levels of the rivers Morava, Thaya and Danube in the east of Austria during 2022 were generally extraordinarily low and the few moderate flood peaks in April, July, August and September did not lead to the environmentally appreciable floodwater due to the high hygroscopicity of the desiccated soil that already showed desiccation cracks. Thus, potential water basins were not filled with water or dried out rapidly before mosquito development was finished. Some abandoned channels of Morava, important biotope not just for mosquitoes but also for freshwater bivalves and snails and many other organisms dried out and accordingly lost their connexion to the main stream. Beside total number of mosquito specimens collected, the composition of floodwater mosquitoes (*Aedes spp.*), house mosquitoes (*Culex spp.*) and other less common mosquitoes (*Anopheles spp.*) as well as the diversity of the trapped (by means of CO₂ traps and ovitraps) and identified species is influenced by these very dry weather conditions especially during the spring and summer months. Monthly (May until end of October), twelve CO₂ traps were used within nine municipalities, additionally ovitraps (one to five ovitraps in three municipalities with different field conditions) and environmental observations were done at least weekly and in close collaboration with the volunteers. On some monitoring dates, on some trap locations (for instance the months May, August and September) the number of mosquitoes tends towards zero. Despite the drought of this year, in some areas (Drösing; May - August) were many mosquito larvae due to targeted, planned flooding by a forestal company (without heads-up) whereas in other areas (Engelhartstetten, Marchegg, Angern) even after flood events in May/June, August and September/October no larvae could be found because the flooded areas dried out very fast. Thus, even in very dry years with nearly no flood events at the (main) rivers standard mosquito surveillance (CO₂ traps, ovitraps) and environmental observations for the estimation of expected larvae productivity of potential breeding sites and following larviciding interventions are reasonable to fix the mosquito number within an acceptable range. Without the above-mentioned observations and controls we could have overlooked the high number of mosquito larvae in one municipality and that would have led to an unacceptable number of adult mosquitoes within the village.

Entomological investigations before and after initiation of mosquito control in Forshaga municipality, Sweden

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Floodwater mosquitoes are a main cause of mosquito nuisance in several European countries, including Sweden. Since 2002, a large-scale mosquito control program around the river Dalälven in eastern Sweden targets floodwater mosquito species such as *Aedes sticticus*. The current study was initiated due to citizen complaints of nuisance mosquitoes in Deje village, Forshaga municipality, a region situated at the river Klarälven in western Sweden. Mosquito collections were performed with CDC-light traps in 2010-2012 to identify the nuisance causing mosquito species and to describe the seasonality, abundance, and species composition of mosquitoes in the area. Over the three years of collections, mosquito abundance increased with a mean of 282 mosquitoes collected per trap-night in 2010, 702 mosquitoes per trap-night in 2011, and 1,367 mosquitoes per trap-night in 2012, despite a lesser river discharge over the same period. The increase in abundance was mainly due to an increase of floodwater mosquitoes, with *Ae. sticticus* as the main species. Therefore, mosquito control with VectoBac G was initiated and performed in 2020. Here, we will present the entomological investigations performed in Forshaga municipality before and after mosquito control.

Non-target insects in wetlands with and without mosquito control in the River Dalälven floodplains, central Sweden

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In Sweden, Bti-based floodwater mosquito control was commenced in 2002 in the River Dalälven floodplains. Bti may have some effects on non-target fauna and this motivated a long-term follow-up study on non-target organisms in six wetlands. The initial follow up study could not detect any negative effects neither on abundance nor diversity of insects. Since small effects may have been missed, an improved design with 12 wetlands, six experimental (treated) and six reference (untreated) areas, commenced in 2012. The results on wetland insect fauna composition and weekly abundance for the first eight years, 2012-2019, showed again complete lack of detectable negative effects at any taxonomic level from order to family and that hydrology was the main structural factor for the insect fauna. However, we did observe a significant abundance difference for the sub-family Chironominae between reference and experimental areas. A closer look at the data showed a strong increase of Chironominae in the reference areas without a simultaneous increase in the experimental areas. Our final interpretation of the results is still pending, and we appreciate input from the audience.

Malaria - the risk of the re-emergence in Romania

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Malaria is a worldwide spread disease, millions of people being affected by it every year in Africa, India, South-East Asia, the Middle East, Central and South America, which means that almost 50% of the world's population is at risk of being infected with malaria. Three factors need to coexist for malaria to re-emerge in Romania: the presence of the *Anopheles* vector species, the circulation of the malaria agent in nature, as well as the occurrence of favourable climatic factors. The study has followed the identification by using the PCR (Polymerase Chain Reaction) of the members of the *Anopheles maculipennis* complex from the North-eastern area of Romania from the city of Iași and from Danube Delta. In total there have been identified by using the PCR amplifying the ITS2 sequence of the ribosomal DNA, 217 specimens belonging to the complex of *An. maculipennis* among which: 58 *An. atroparvus*, 18 *An. melanoon*, 2 *An. labranchiae*, 52 *An. maculipennis* and 87 *An. messeae*. *Anopheles labranchiae* is the most important vector of malaria in Europe and *P. falciparum* can develop experimentally in these mosquitoes. Between 1967, when malaria has been completely eradicated in our country, and present, the temperatures increased by 1°C. The presence of vectors, the introduction of parasites and the conducive climate raise questions about the possibility of malaria re-emerging and becoming re-established in Romania. Using a mathematical model that we have implemented, which is based on the construction of a Lagrange polynomial interpolation function, we have realised an extrapolation of the temperature evolution for the year 2100, suggesting the progression of some favourable conditions for the development of the *Anopheles* vector species and of the malaria parasite inside of it.

Keywords: malaria, re-emergence, mosquitoes.

Best practice for wetland mosquito control in Sweden

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The river Dalälven flows through central Sweden and is known for its high biodiversity since the northern and southern flora and fauna meet here. During spring and occasionally in summer, major flooding events produce enormous amounts of floodwater mosquitoes that cause a nuisance for the people in this area. Daily monitoring of water levels starts in April through field visits and installation of hydrostatic probes in risk areas. Based on the flooding magnitude and the abundance of floodwater mosquito larvae, an evaluation is made for the need of a treatment. Inventory of larvae is measured with a dipper and requires, according to regulations, more than 4 larvae per liter in relevant areas. The water edge of the flooded area is measured by GPS and analysed with a digital elevation model in GIS (Geographical Information Systems). Digital maps are sent to a helicopter to implement the airborne treatments with granules of the biological control agent VectoBac G[®], containing *Bacillus thuringiensis israelensis* (Bti). The treated areas are visited 24 hours post treatment to make a new inventory of larvae, which often results in finding only dead larvae.

Time is running: How to assess *Aedes albopictus* abundance and operational efficiency of vector control in an outbreak context in Europe? Case studies of outbreaks in France from 2020 to 2022.

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Metropolitan France has the highest frequency of events of autochthonous transmission of dengue, chikungunya and Zika in continental European area, with at least 31 independent events from 2010 to 2022. Once an autochthonous case is detected in France, rapid curative vector control operations are implemented: entomological investigations, population information and insecticide treatments must be realized within 3 working days. This leaves little time to estimate the vector situation before treatment. These data are thus necessary a/ to better understand the entomological and epidemiological conditions necessary for an outbreak in a temperate environment and b/ to evaluate the effectiveness of mosquito control interventions. We use two entomological indicators when possible: 1/ Larval (*Stegomyia*) indices, considering the larval abundance (with the abacus method) and 2/ adult mosquito trapping. Based on case studies of autochthonous dengue events that occurred in France between 2020 and 2022, we compare both methods and present the value and limits of these methods in function of field conditions: time between viremia and mosquito control intervention, urban fabric, vector seasonality etc. Our experience brings arguments to develop guidelines of standardized vector density indicators to allow the comparison of outbreaks in Europe and in temperate environments.

Conceptualization and logistics of the traps distribution phase of the Citizen Science project NESCOTIGER: New strategies for the control of *Aedes albopictus* in residential areas

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The invasive mosquito *Aedes albopictus* is currently widespread in European countries. Control activities deployed by the authorities become only partially effective due to the presence of domestic private breeding sites. Mass trapping appears to be a promising solution with limited environmental impact but need intensive citizen collaboration. In this sense, the NESCOTIGER project, “New strategies for the control of the tiger mosquito in residential areas”, was designed and executed in the residential area of El Vedat de Torrent (Valencia, Spain). Four trapping approaches were evaluated in the study site in five sectors defined in a total area of 434.7 ha for mass control of mosquitoes in residential areas. Residents were asked to collect, place, and maintain the traps in their gardens, for which general information was available through different means. Mosquito traps were distributed by five public services and neighborhood associations between April and July 2022. We describe here the work behind the conceptualization and development of the database and its functionality developed for the control tools distribution phase. Firstly, a spatial risk analysis was conducted considering potential breeding sites of *Ae. albopictus*, housing, street catch basins and vegetation density. Based on this analysis, the five sectors were delimited, and the total number of households and surface parcel size was registered. Secondly, based on the plot size of each household, quantiles were calculated for each sector, each getting a specific trap amount. A range of one to four units corresponded to each household based on the residence’s size. A specific data sheet was created containing this information and was sent to the different distributing sites. Distributors were trained in the operation of this data sheet, and a grand total of 1247 traps were distributed during the four-month period . The same process could be employed in future similar research projects.

Entomological surveillance of urban areas in the West Nile virus outbreak area in Seville and Malaga (Andalusia, Southern Spain): Period 2021-2022

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Since August 2020, an outbreak of human West Nile virus (WNV) has been active in the autonomous communities of Extremadura (Western Spain) and specially Andalusia (Southern Spain). In the former, the autonomous public services in response to this outbreak established the mandatory development of Entomological Surveys in every municipality. The present study aims to review the results of the Entomological Survey of ten different municipalities in the provinces of Seville and Malaga (Andalusia) carried out between May 2021 and August 2022 (still ongoing during September and October 2022). A total of two to five BG-Sentinel 2 traps, baited with BG-Lure attractant, were set in each municipality. Traps were kept constantly operating and were replaced once or twice every month (depending on the established contract with each municipality). A total of 12098 mosquitos were captured, sexed and identified to species levels. Also, in the case of females, individuals were classified as unfed, blood-fed or gravid. Eight different species belonging to four different genera were identified: 75.5% *Culex* (*Cx. pipiens*, 45.6%; *Cx. perexiguus*, 26.8%; *Cx. theileri*, 3.08% and *Cx. modestus*, 0.02%), 13.1% *Anopheles* (*An. maculipennis*), 7.60% *Aedes* (*Ae. albopictus*, 7.22%; *Ae. caspius*, 0.39%) and 3.79% *Culiseta* (*Cs. longiareolata*). Distribution maps and phenological graphics are developed in a ten-time period since the collection of the traps. Based on those results, control campaigns are modulated and developed. *Culex* species are the most abundant in the study area, specially *Cx. pipiens* and *Cx. perexiguus*, both main vectors and involved in the transmission of WNV, either mainly in urban or rural areas respectively. As traps were set in urban or periurban areas, higher capture rates of urban mosquitoes such as *Cx. pipiens*, *Ae. albopictus* or *Cs. longiareolata* were to be expected. Nevertheless, other more rustic mosquitoes such as *Cx. perexiguus*, *An. maculipennis* or *Cx. theileri* point to the introduction of rural mosquitoes into urban environments from untreated breeding sites, mostly rice fields and other floodable areas.

Surveillance of mosquito larvae in the Valencian Autonomous Region (Eastern Spain)

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Valencian Autonomous Region, due to its geographical location and its geological typology, is characterized by the presence of numerous marshes along its Mediterranean coastline. These flooded environments provide an optimal habitat for various species of mosquitoes of several genera. So much so that this region was in the past an endemic area for diseases such as malaria. The Entomology and Pest Control Laboratory of the University of Valencia has been studying the diversity of mosquitoes in this area of Spain for more than 40 years. As a consequence of this extensive experience, and due to the increasing notification of imported cases of arboviruses such as dengue, zika, chikungunya and West Nile, the General Directorate of Public Health and Additions of the Valencian Autonomous Government decided in 2016 to form a multidisciplinary work group with the objective of joining forces to safeguard the health of citizens. Since then, this Laboratory has been in charge of carrying out the entomological surveillance of autochthonous and non-native mosquitoes such as the tiger mosquito, to find out their urban, peri-urban and natural distribution, the population sizes, as well as transferring this information to the aforementioned general management and to the public health technicians of each municipality advising on where to carry out a treatment, what product and frequency to use, and checking its effectiveness after its application. In this way, it has been possible to know with accuracy the current distribution of vector species such as *Aedes albopictus* or *Culex pipiens*, in this region. To this end, appropriate techniques have been used for each casuistry to inspect mosquito larvae in the different habitats according to the species, which have included dendrotelmas, temporary or permanent marshes, riverbanks, ornamental fountains, pools of irrigation, troughs, scuppers, and many types of garden elements that allow the water accumulation.

Time is running: How to quickly estimate larval abundance of *Stegomyia* mosquitoes with Abacus method

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Stegomyia mosquitoes (*Aedes albopictus* and *Ae. aegypti*) are important vectors of arboviruses. During outbreaks, larval indices are the reference, but lack of time do not currently allow for an operational estimation of larval densities. Faster and more accurate population abundance assessment methods need to be developed to improve vector surveillance and the evaluation of the effectiveness of vector control interventions by operators. The abacus method has been adapted to the container mosquitoes to allow a rapid visual estimation of larval abundance on site. The abacus method consists of a visual comparison between the abundance of larvae placed in a standardized 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. Our main objective is to test the reliability and efficiency of the abacus method initially developed for marsh mosquitoes and validate this method as a complement to traditional larval indices. The influence of several factors on these readings was analyzed: 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 1,800 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). Errors in species identification caused extreme estimation errors. The time required to use the abacus method is much shorter than the larvae-by-larvae counting method and thus saves a considerable amount of time. The abacus method is suitable for widespread application in the field for container-breeding mosquito species.

Best practices for detecting invasive mosquitoes at Points of Entry

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Since the Asian tiger mosquito has been involved in local transmission of mosquito-borne diseases in Europe, active surveillance for invasive mosquito species (IMS) has become of the utmost importance.

The main sites at risk for introduction of IMS are the so-called Points of Entry (PoE), e.g., storage sites for imported used tyres, greenhouses for imported aquatic plants, international airports, container terminals, highways, and road axes that originate in colonized areas. Several approaches can be applied for the surveillance at the PoE. Each of them has different costs and chances of success. After a decade of surveillance at PoE, we report our observations on the main methods applied in Piedmont (Italy): eggs collected by ovitraps, adults collected by BG-Sentinel traps, and larvae collected by breeding-sites sampling. Ovitrap are low-cost and easy to use, but egg identification requires particular skills and equipment. Morphological identification of the late larval instars obtained by hatching out eggs or adults obtained by rearing the larvae is much easier. In the last three years (2019-2021), a total of 581 masonite strips (used as oviposition substrate) were placed in ovitraps at PoE, collected and submerged to obtain larvae. The larvae were later reared to the adult stage, obtaining only *Aedes albopictus* and *Aedes geniculatus* specimens. In the same period, from a total of 199 BG-Sentinel trapping sessions at PoE, only an IMS specimen (*Aedes japonicus*) was captured (0.5%).

Larval search consisted in search and sampling immature mosquito stages, particularly L3-L4 larvae and pupae, on small man-made water containers that accumulate dead leaves, algae and/or other organic matter. This kind of inspections often involved the areas surrounding the PoE, focusing on sites that offer conditions conducive to the development of mosquitoes, e.g., cemeteries. Larval samples collected from 126 different sites were reared, obtaining IMS specimens (*Ae. japonicus* and *Aedes koreicus*) from 16 sites (13%).

Undoubtedly, larval search takes longer than other methods, but this method allowed us to obtain a greater amount of data on introduction and spread of IMS.

Evaluation of the manhole physical pest management system UNFO-PLS for mosquito control in southern Switzerland.

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The container-breeding invasive exotic mosquito *Aedes albopictus* has spread rapidly over the last few decades across Europe. To limit the effects of the presence of this mosquito species, several control systems have been in place in Europe for years to control its density. Integrated vector management (IVM) has been implemented to control its numbers. Here, we evaluated the efficacy of one of the mechanical systems available on the market that aim to prevent mosquitoes from gaining access to manhole water, thus preventing reproduction in these receptors. SUPSI collaborated in the development and subsequently tested one of these systems proposed by the Italian company UNFO PLS s.r.l. (<https://www.unfo-pls.com>).

The experiment was done in the municipality of Balerna, Switzerland. Three study zones were selected, each containing four experimental and four control manholes. The UNFO Pest Lock System was placed in the experimental manholes and compared to the control manholes without the device during 2020 and 2021. This allows us to compare the differences in the presence and abundance of mosquito breeding container larval stages to determine the effectiveness of the mechanical system.

Mosquito counts at all developmental stages were significantly lower at manholes fitted with UNFO-PLS devices compared to control manholes. The percent reduction in mosquito counts at the L1 stage was 95.2%, at the L2 stage was 96.3%, at the L3 stage was 97.2%, at the L4 stage was 95.0% and at the pupal stage was 92.6%.

UNFO-PLS devices are highly effective at reducing the presence of L1-L4 stage and pupal stage mosquitos in sewer system drainage holes present in Balerna. The use of this tool is interesting in an integrated *Ae. albopictus* control system. However, the cost-benefit ratio against the use of the larvicides must be evaluated.

Distribution and spread of *Aedes koreicus* in the city of Wiesbaden, Germany (2019-2021)

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The Korean Bush Mosquito *Aedes koreicus* is native to Korea, north-eastern China, eastern Russia and parts of Japan. It was found for the first time outside of its native range in 2008 in Belgium. After this record it appeared in several European countries in the following decade. In Germany, *Ae. koreicus* was first discovered in 2015 near Augsburg, Bavaria, and in 2016 in the city of Wiesbaden, Hesse. In the latter place it started to build up an established population. From 2019 to 2021 this population was monitored in different parts of the city and the surrounding areas. Altogether, 36 locations were investigated by ovitrap sampling mainly on cemeteries. Additional larval sampling was performed in vases and basins.

The species showed only a slow expansion and after three years the populated area was just about 150 % compared to the time before this investigation. This might be due to topographic issues and land use but also to the simultaneous arrival and spread of *Ae. j. japonicus* which was very abundant in the mountainous areas around the city. Both species are now present in almost all parts of the town and also in a small area across the river Rhine in the city of Mainz.

Mass trapping and larval source management for mosquito elimination on small Maldivian islands

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Globally, environmental impacts and insecticide resistance are forcing pest control organisations to adopt eco-friendly and insecticide-free alternatives to reduce the risk of mosquito-borne diseases, which affect millions of people, such as dengue, chikungunya or Zika. We used, for the first time, a combination of human odor-baited mosquito traps (at 6.0 traps/ha), oviposition traps (7.2 traps/ha) and larval source management (LSM) to practically eliminate populations of the Asian tiger mosquito *Aedes albopictus* (peak suppression 93.0% (95% CI 91.7–94.4)) and the Southern house mosquito *Culex quinquefasciatus* (peak suppression 98.3% (95% CI 97.0–99.5)) from a Maldivian island (size: 41.4 ha) within a year and thereafter observed a similar collapse of populations on a second island (size 49.0 ha; trap densities 4.1/ha and 8.2/ha for both trap types, respectively). On a third island (1.6 ha in size), we increased the human odor-baited trap density to 6.3/ha and then to 18.8/ha (combined with LSM but without oviposition traps), after which the *Aedes* mosquito population was eliminated within 2 months. Such suppression levels eliminate the risk of arboviral disease transmission for local communities and safeguard tourism, a vital economic resource for small island developing states. Terminating intense insecticide use (through fogging) benefits human and environmental health and restores insect biodiversity, coral reefs and marine life in these small and fragile island ecosystems. Moreover, trapping poses a convincing alternative to chemical control and reaches impact levels comparable to contemporary genetic control strategies. This can benefit numerous communities and provide livelihood options in small tropical islands around the world where mosquitoes pose both a nuisance and disease threat.

Pilot project of the biological control of mosquitoes in the territory of the city of Bratislava - significant changes during the 2021 and 2022

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In the pilot project of the biological control of mosquitoes in the territory of the city of Bratislava, significant changes of monitoring strategy and BTI application efficiency tests took place in 2021 and 2022. Implementation of new methodology of the biological control of mosquitoes led to the expansion of the implementation area.

The work evaluates several goals that need to be achieved for the overall effectiveness of joint activities. The first parameter concerns the necessary frequency of monitoring in order to obtain relevant information about the occurrence of mosquito larvae in the urban environment and in the city outskirts.

Secondly, operational capacities of personnel need to be evaluated in order to determine the maximal area that can be monitored or treated with various types of interventions. The work focuses on the comparison of the year-on-year personnel effectiveness (volunteers and professional workers) depending on training between 2021 and 2022.

Furthermore, the study evaluates the number of BTI applications performed and their time consumption in different types of breeding sites (eg. rainwater catch basins) according to the BTI application method used. It elaborates the impact of the adult mosquito population on the ratio of the monitored areas and areas where monitoring or interventions are not allowed.

It evaluates the time-effectiveness of monitoring breeding sites and of different methods of BTI application in the most critical areas of the city with a proposal to create clusters of breeding sites and monitoring routes.

Finally, it describes the elements of the newly created mobile application as a data centre for sharing information between all involved parties including cross-border cooperation INTERREG Slovakia – Austria.

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