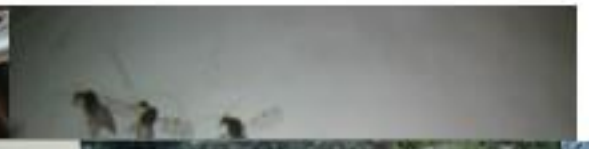
# Barcelo Carlos, University of the Balearic Islands, (ES)

# From monitoring to pathogens and host detection of *Ae. albopictus* in the Balearic Islands

**Carlos Barceló**, Rafael Gutiérrez-López, Mikel. A. González, Stephanie Jansen, Konstantin Kliemke, Jonas Schmidt-Chanasit, Miguel Ángel Miranda & Renke Lühken

AIM-COST conference, 1-2 February 2023, Rome

# Before the AIM-COST Short-term Scientific Mission





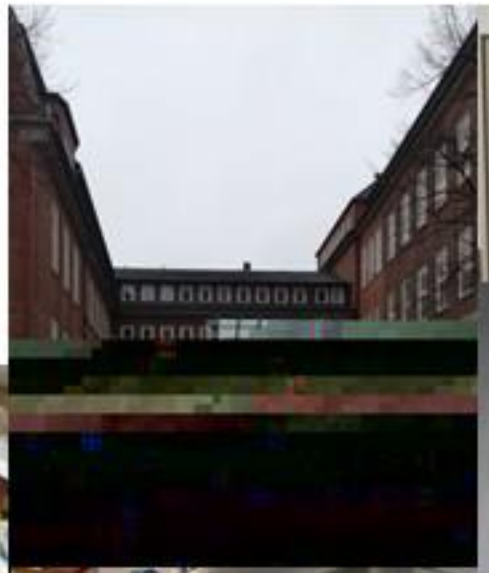




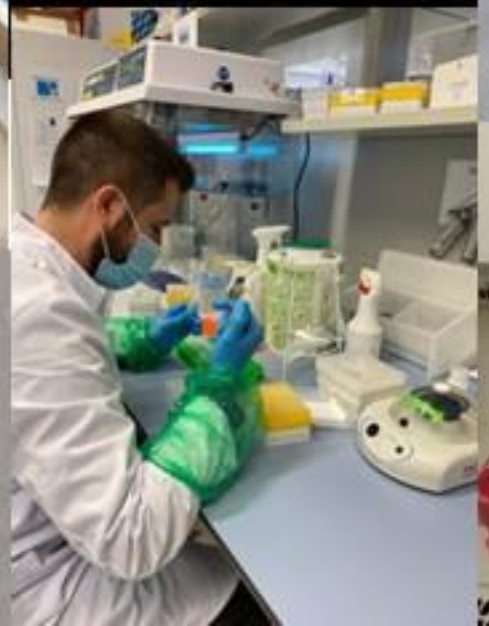

# During the AIM-COST Short-term Scientific Mission

3 x Urban parks
4 x Peri-urban areas
8 x Rural areas (livestock farms)
5 x Natural areas
1 x Dog pound

Total: 16,312 mosquito individuals + 383 engorged females







Engorged females:
5% avian malaria (+) at species level
1.3 % *Dirofilaria* (+) at species level
Non-engorged females:
1.5 % (+) at some pathogen

# After the AIM-COST Short-term Scientific Mission

## Two projects

*Study of the prevalenceof arboviruses and other pathogens transmitted by diptera in livestock farms in the Balearic Islands*

GOIB
CONSELLERIA AGRICULTURA, PESCA I ALIMENTACIÓ
FONS GARANTIA AGRÀRIA I PESQUERA ILLES BALEARS

BNITM
Bernhard Nocht Institute for Tropical Medicine

*An open-access wing-base to allow the species identification of mosquitoes in Europe*

## New tools/new techniques/new ideas

European Mosquito Control Association

# XI$^{th}$ International Conference

**7-10 November 2023, Palma, Spain**

# SHAPING THE FUTURE OF VECTOR CONTROL IN EUROPE

# Belavilas Trovas Alexandros, University of Thessaly (EL)

# Disruption of the reproductive capacity of *Aedes* mosquitoes by targeting lncRNAs

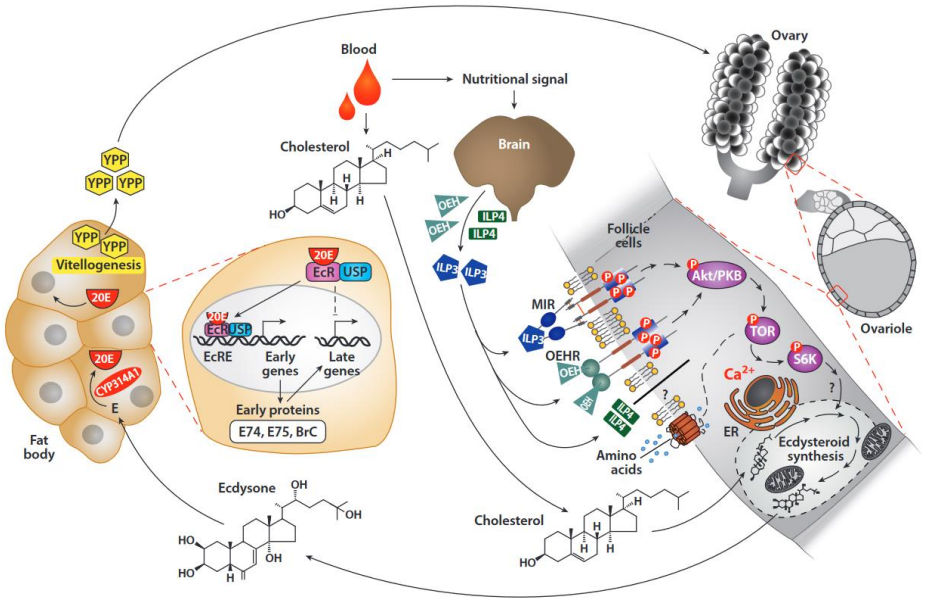
**Alexandros Belavilas-Trovas**
**PhD fellow**

DEPARTMENT OF Biochemistry & Biotechnology
UNIVERSITY OF THESSALY

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

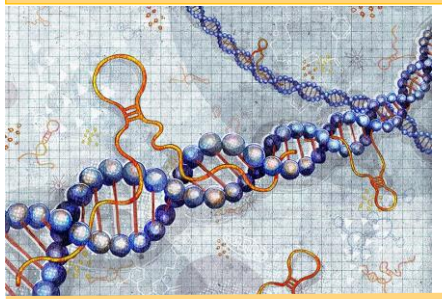
*Ae. albopictus*

*Ae. aegypti*




Signaling pathways of vitellogenesis (Roy *et al* 2018)

## Long non-coding RNAs (lncRNAs)



- Transcripts >200nt
- No coding potential
- Regulators of biological pathways
- Species-specific

➢ **Promising tools for species-specific control approaches**

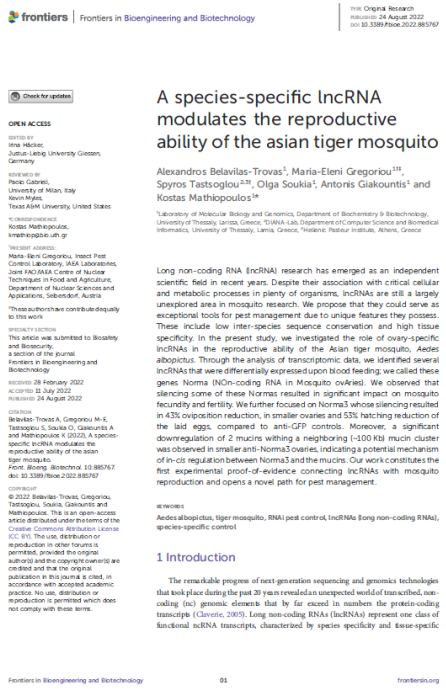
# *Impact of STSM on scientific growth & career*

1 Basic research

2 Applicability

3 Infrastructure

4 Engagement



frontiers | Frontiers in Bioengineering and Biotechnology

TYPE Original Research
PUBLISHED 24 August 2022
DOI 10.3389/fbioe.2022.885767

Check for updates

OPEN ACCESS

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‡These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Biosafety and Biosecurity, a section of the journal Frontiers in Bioengineering and Biotechnology

RECEIVED 28 February 2022
ACCEPTED 18 July 2022
PUBLISHED 24 August 2022

CITATION
Belavilas-Trovas A, Gregoriou M-E, Tastsoglou S, Soulka O, Giakountis A and Mathiopoulos K (2022). A species-specific lncRNA modulates the reproductive ability of the asian tiger mosquito. *Front. Bioeng. Biotechnol.* 10:885767. doi: 10.3389/fbioe.2022.885767

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A species-specific lncRNA modulates the reproductive ability of the asian tiger mosquito

Alexandros Belavilas-Trovas[1], Maria-Eleni Gregoriou[1†‡], Spyros Tastsoglou[2,3‡], Olga Soulka[1], Antonis Giakountis[1] and Kostas Mathiopoulos[1]*

[1]Laboratory of Molecular Biology and Genomics, Department of Biochemistry & Biotechnology, University of Thessaly, Larissa, Greece, [2]DIANA-Lab, Department of Computer Science and Biomedical Informatics, University of Thessaly, Lamia, Greece, [3]Hellenic Pasteur Institute, Athens, Greece

Long non-coding RNA (lncRNA) research has emerged as an independent scientific field in recent years. Despite their association with critical cellular and metabolic processes in plenty of organisms, lncRNAs are still a largely unexplored area in mosquito research. We propose that they could serve as exceptional tools for pest management due to unique features they possess. These include low inter-species sequence conservation and high tissue specificity. In the present study, we investigated the role of ovary-specific lncRNAs in the reproductive ability of the Asian tiger mosquito, *Aedes albopictus*. Through the analysis of transcriptomic data, we identified several lncRNAs that were differentially expressed upon blood feeding; we called these genes Norma (NOn-coding RNA in Mosquito ovAries). We observed that silencing some of these Normas resulted in significant impact on mosquito fecundity and fertility. We further focused on Norma3 whose silencing resulted in 43% oviposition reduction, in smaller ovaries and 53% hatching reduction of the laid eggs, compared to anti-GFP controls. Moreover, a significant downregulation of 2 mucins withing a neighboring (~100 Kb) mucin cluster was observed in smaller anti-Norma3 ovaries, indicating a potential mechanism of in-*cis* regulation between Norma3 and the mucins. Our work constitutes the first experimental proof-of-evidence connecting lncRNAs with mosquito reproduction and opens a novel path for pest management.

KEYWORDS
*Aedes albopictus*, tiger mosquito, RNAi pest control, lncRNAs (long non-coding RNAs), species-specific control

## 1 Introduction

The remarkable progress of next-generation sequencing and genomics technologies that took place during the past 20 years revealed an unexpected world of transcribed, non-coding (nc) genomic elements that by far exceed in numbers the protein-coding transcripts (Claverie, 2005). Long non-coding RNAs (lncRNAs) represent one class of functional ncRNA transcripts, characterized by species specificity and tissue-specific

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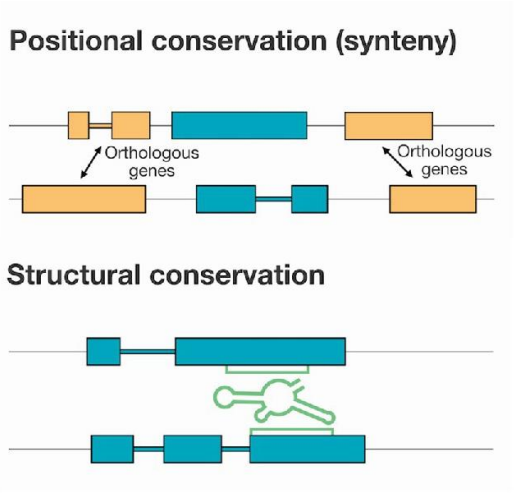
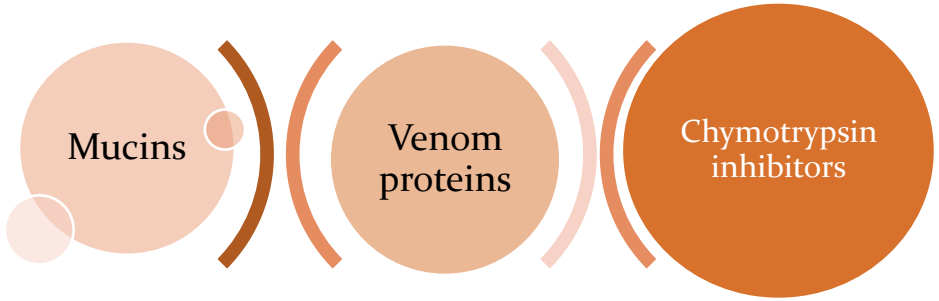



- A conservation model between two divergent reproductive lncRNA genes of *Ae. aegypti* & *Ae. albopictus*



# *Impact of STSM on scientific growth & career*

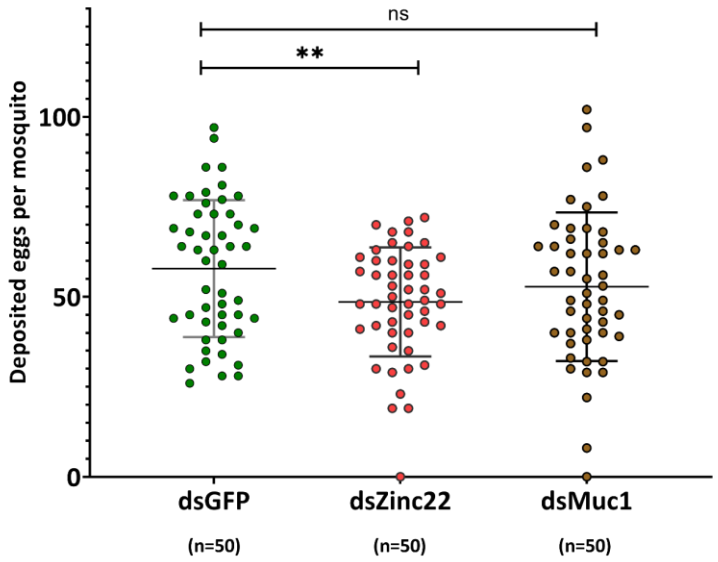
1 Basic research

2 Applicability

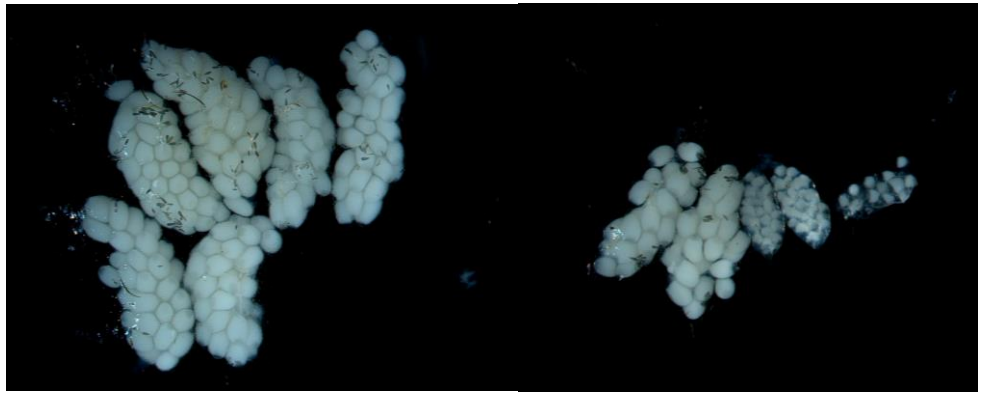
3 Infrastructure

4 Engagement

- Reduced fecundity




- Provoked smaller ovaries



Control Treatment

# *Impact of STSM on scientific growth & career*

1 Basic research

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*Ae. aegypti*


Microinjector

- Joined a divergent, multicultural environment
- Prepared the ground for broader collaborations

# Acknowledgements


DEPARTMENT OF Biochemistry & Biotechnology UNIVERSITY OF THESSALY

- Prof. Kostas Mathiopoulos (supervisor)

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

- Prof. Marc Schetelig (host)
- Dr. Irina Häcker

Bourquia Maria, Institut Agronomique et Vétérinaire Hassan II, Rabat, (MA)

# Vector role of mosquitoes in the mediterranean region : identification and field monitoring methods of native and AIM species in urban, rural and natural areas


Maria Bourquia

*DVM, PhD*

*Parasitology and Parasitic diseases Unit*

*Institut Agronomique et vétérinaire Hassan II, Rabat, Morocco*

m.bourquia@iav.ac.ma


01/02/2023

# STSM

**Why applying to STSM ?**

PhD thesis 2016-2019 : Environmental drivers of the distribution of *Culicoides* (Diptera: Ceratopogonidae), vectors of viruses of economic interest, in Morocco

- Strengthen my competences for other vector groups, in particular mosquitoes

**STSM:**

Date : 02/09/2021 to 16/09/2021

Place : Applied zoology and animal conservation research group (ZAP) University of Balearic Islands of Palma de Majorcan Spain

Purpose :

-Training of field monitoring methods of native and AIM species and other vector insects in urban, rural and natural areas

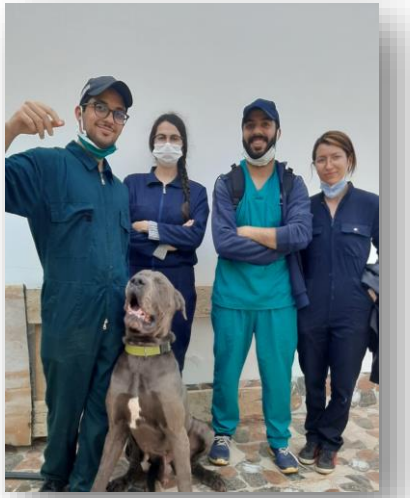
-Advanced training in morphological identification of mosquito vectors in the Mediterranean region

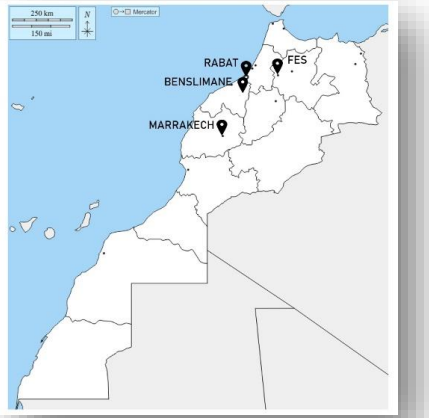
# STSM CONTRIBUTION TO MY RESEARCH

- Consolidate and strengthen my knowledge and skills in field monitoring, systematics and taxonomy of mosquito of veterinary and medical interest


- Study : Characterization of mosquito fauna at the zoological garden of Rabat, Morocco (ESOVE congress, Sofia, Bulgaria /article under prep)

- PhD thesis project about arthropod vectors (including mosquitoes) in dog shelters in four Moroccan regions






- Collaborations are underway with ZAP group on different topics (trainings and exchanges)

Applied Zoology and Animal Conservation Research Group (ZAP-UIB)

# Acknowledgment

Funded by the European Union

AIM
Aedes Invasive Mosquitoes

cost
EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

Applied Zoology and Animal Conservation Research Group (Z4P-UIB)

# Da Re Daniele, University of Trento, (IT)

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fnrs
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UNIVERSITÀ DI TRENTO

C3A
CENTRO AGRICOLTURA ALIMENTI AMBIENTE

Edge Hill University

Public Health England

# Learning in the field:

# improving the realism of mechanistic models forecast through interactions

# with UK's medical entomologists

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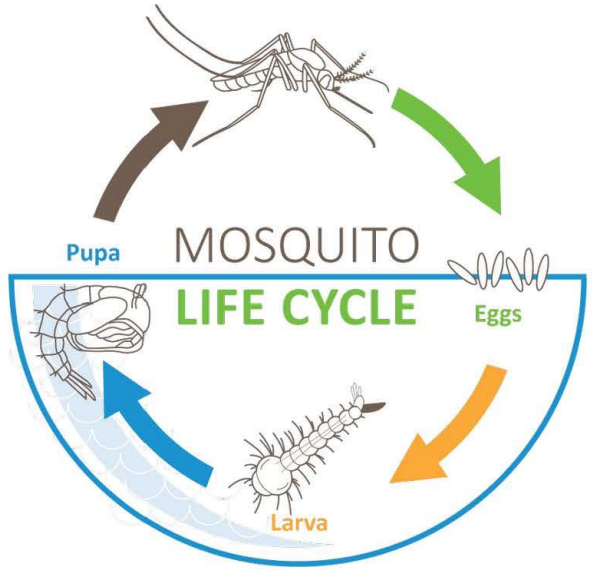
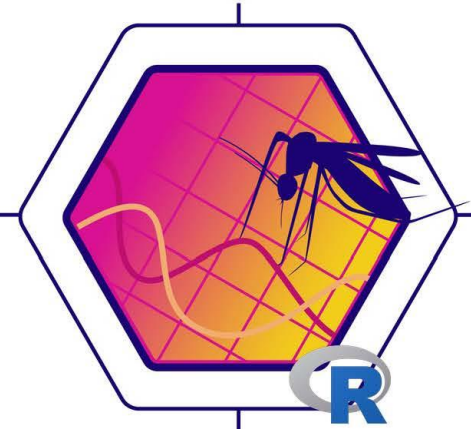
Daniele Da Re – University of Trento (Italy)

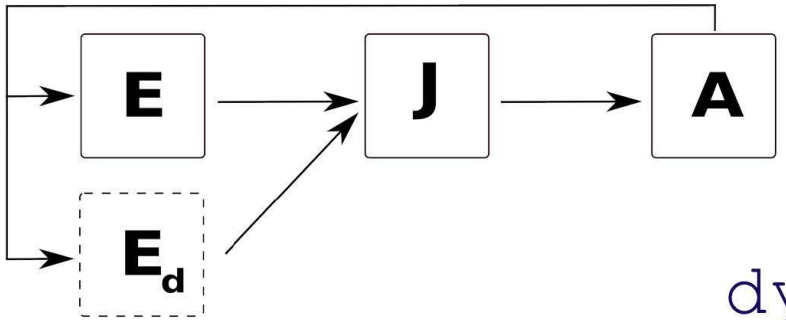



dynamAedes



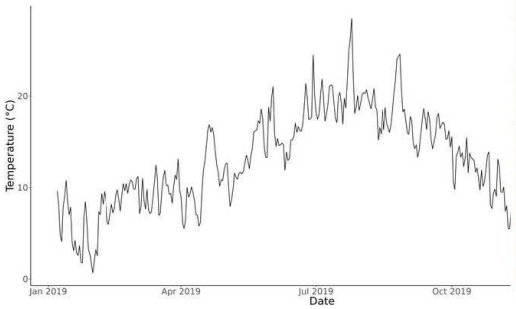
# dynamAedes

## Model structure

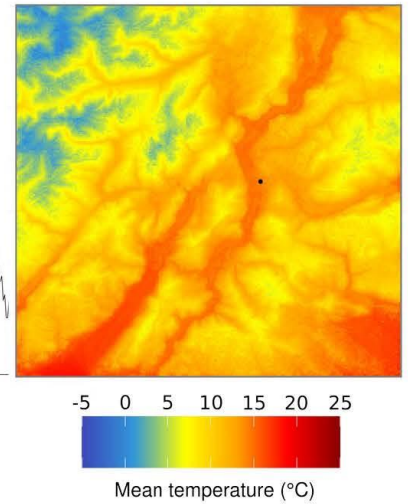



## Input temperature datasets

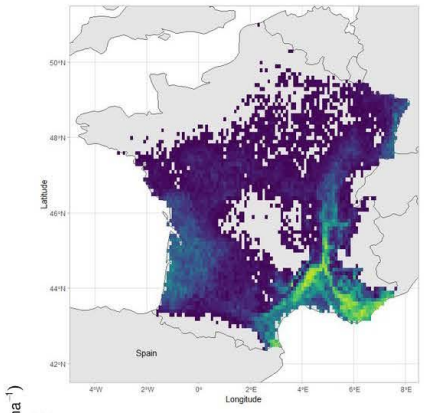
Temporal



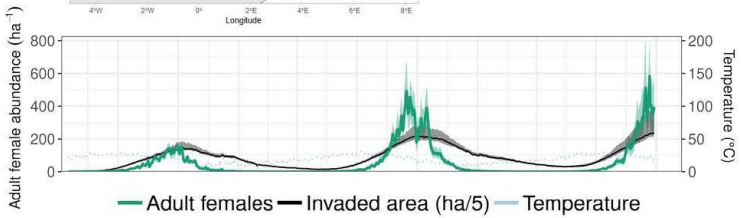

Spatial




## Outputs

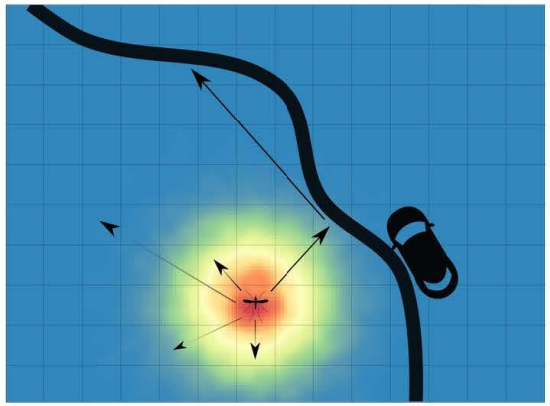



% of successful introduction



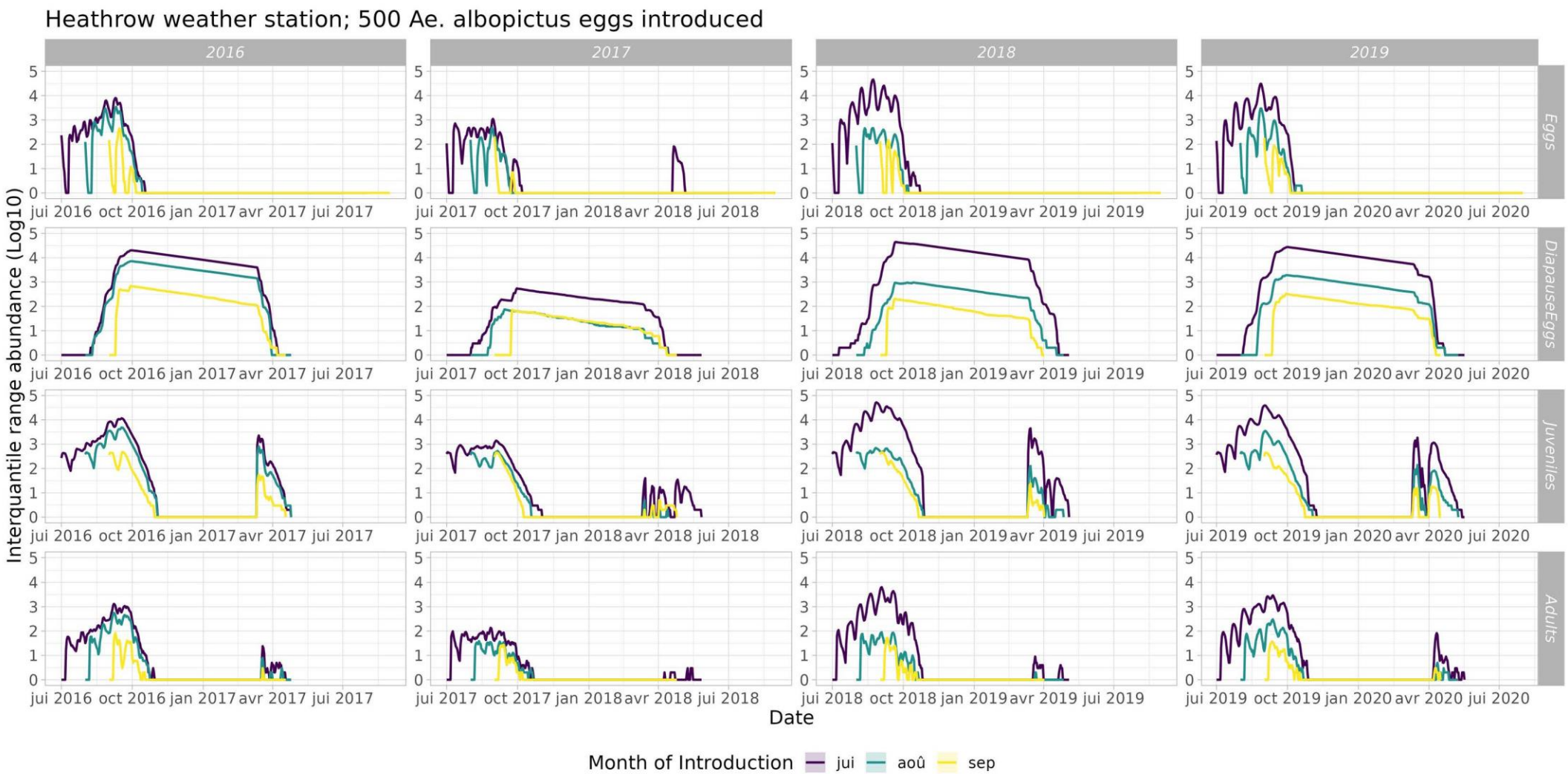

Population dynamics

## Adults' active and passive dispersal



# Simulating the potential overwintering of *Ae. albopictus* in the UK

# Simulating the potential overwintering of *Ae. albopictus* in the UK


Heathrow weather station; 500 Ae. albopictus eggs introduced
2016
2017
2018
2019
Eggs
DiapauseEggs
Juveniles
Adults
Interquantile range abundance (Log10)
0
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oct 2016
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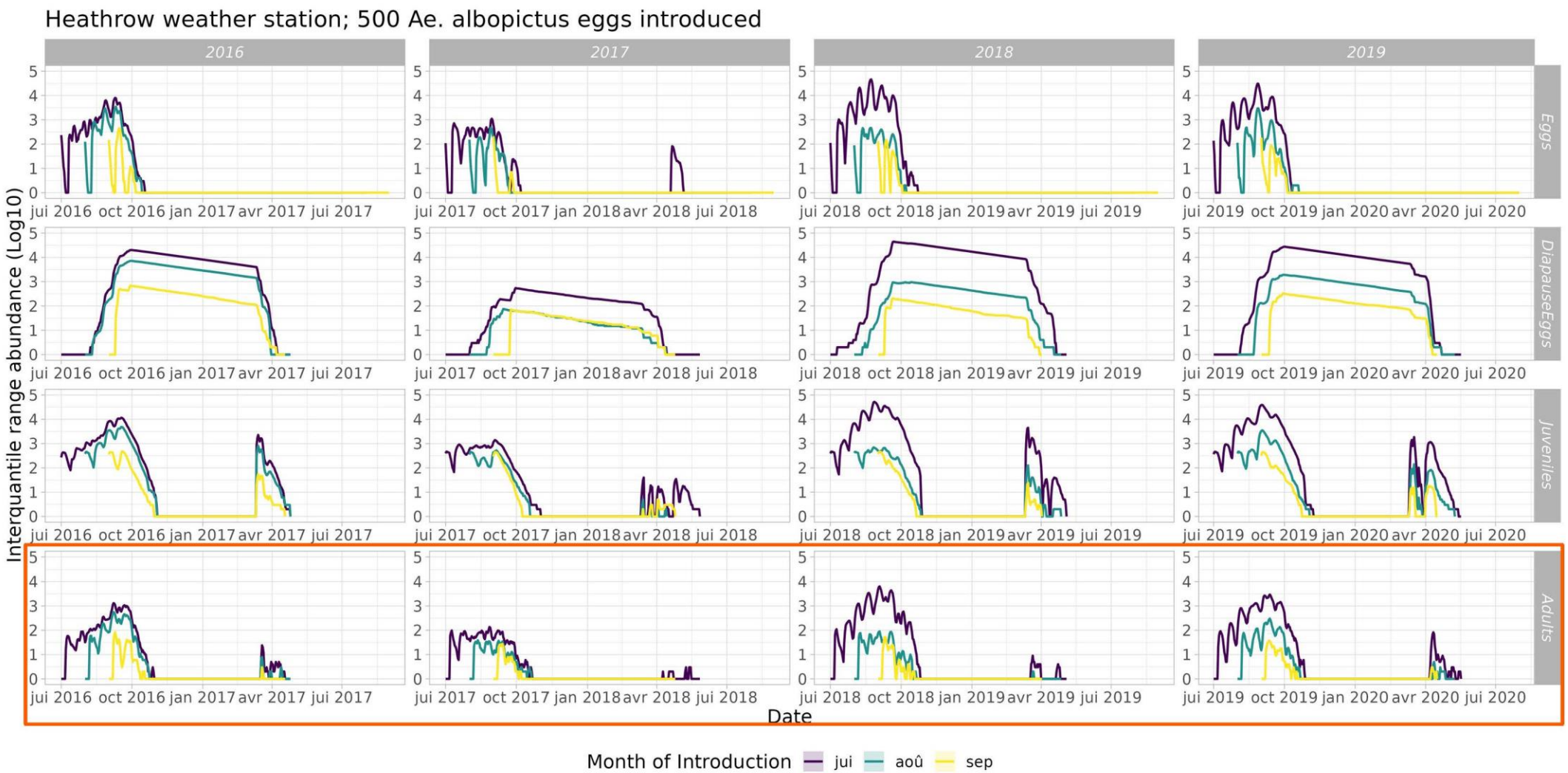
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Date
Month of Introduction
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# *Thank you*

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daniele.dare@unitn.it

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SENCKENBERG world of biodiversity
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AIM Aedes Invasive Mosquitoes
cost EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY
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UNIVERSITÀ DI TRENTO
C3A CENTRO AGRICOLTURA ALIMENTI AMBIENTE
Edge Hill University
THE UNIVERSITY OF QUEENSLAND AUSTRALIA
INSTITUTE OF TROPICAL MEDICINE ANTWERP
Istituto Zooprofilattico Sperimentale delle Venezie
Public Health England

Gonzalez Mikel Alexander, University of the Balearic Islands, (ES)

Mikel Alexander González

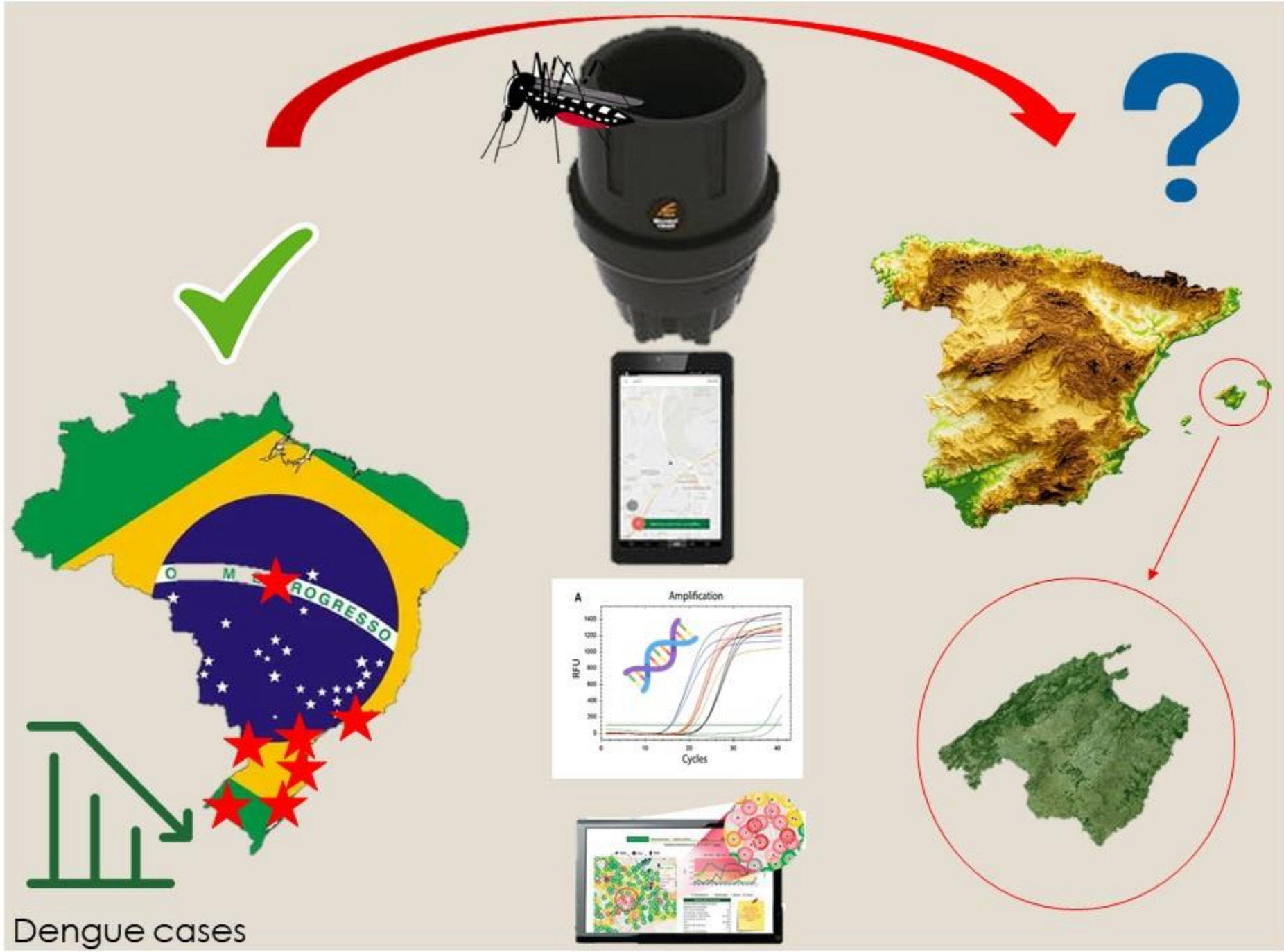
mikel_heredia

# Could an intelligent monitoring technology system developed to combat *Aedes aegypti* in Brazil work for *Ae. albopictus* in Europe?


Universitat de les Illes Balears

ZAP Applied Zoology and Animal Conservation Research Group

Ecovec A Rentokil Company


?
A
Amplification
RFU
Cycles
Dengue cases


Breeding sites are everywhere vs breeding sites are more restricted

In winter, biting activity is uninterrupted in Brazil vs limited in Europe

Cultural practices and waste management differ markedly between both

Vector control is inefficient and delayed vs efficient and rapid in case of outbreaks/high densities

Arbovirus circulation is high in Brazil vs sporadic outbreaks in Europe

I have many reasons to think that the scenario between Europe and Latin America are completely different and thus responses and control actions should be adapted in each case

# Horvat Cinthia, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca (RO)

AIM COST Final Conference
*1-2 February 2023,*
*Rome, Italy*

# Short Term Scientific Missions in Novi Sad and in Lisbon

*Learning & Experiences through AIM COST's STSM programme*

**Horváth Cintia**
*University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania*

# Basic taxonomy for species identification of mosquitoes and maintenance of laboratory breeding mosquito colonies

**University of Novi Sad, Serbia**

STSM start and end date: **28.01.2019/ 08.02.2019**

- **Aims:**

1. Learning about the basic taxonomy of mosquitoes;
2. Maintenance of laboratory breeding mosquito colonies (*Cx. pipiens* biotype *molestus* & *Ae. albopictus*);

- **Outcome:**

1. Learning to identify male and female mosquitoes, larvae;
2. Learning about the rearing procedures;
3. Creating a laboratory suitable for breeding *Ae. albopictus*, and maintaining a colony at the UASVM;

# Microsatellite-based population analysis of *Aedes albopictus* (Diptera: Culicidae) from Romania

**Institute of Hygiene and Tropical Medicine (IHMT)**, Lisbon, Portugal

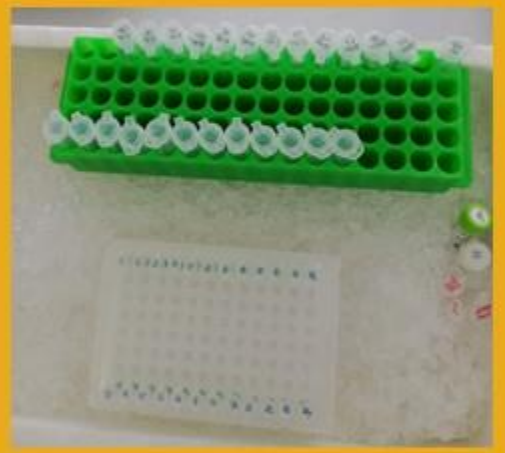
STSM start and end date: **03.05.2021/ 21.05.2021**

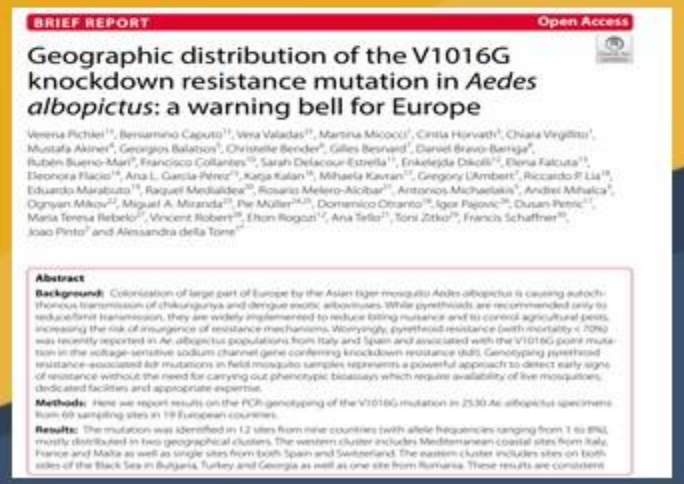
- **Aims:**

1. To learn about the basics of population genetics;
2. To gain experience in microsatellite-based population genetic analysis of invasive mosquito species

- **Outcome:**

1. Learning about the introduction events and phylogenetic relationship among Romanian *Ae. albopictus* populations + identifying pyrethroid resistance (V1016G);
2. 2 research papers;





BRIEF REPORT Open Access

Geographic distribution of the V1016G knockdown resistance mutation in *Aedes albopictus*: a warning bell for Europe

Abstract

**Background:** Colonisation of large part of Europe by the Asian tiger mosquito *Aedes albopictus* is causing autochthonous transmission of chikungunya and dengue exotic arboviruses. While pyrethroids are recommended only to reduce/limit transmission, they are widely implemented to reduce biting nuisance and to control agricultural pests, increasing the risk of insurgence of resistance mechanisms. Worryingly, pyrethroid resistance (with mortality < 70%) was recently reported in *Ae. albopictus* populations from Italy and Spain and associated with the V1016G point mutation in the voltage-sensitive sodium channel gene conferring knockdown resistance (kdr). Genotyping pyrethroid resistance-associated *kdr* mutations in field mosquito samples represents a powerful approach to detect early signs of resistance without the need for carrying out phenotypic bioassays which require availability of live mosquitoes, dedicated facilities and appropriate expertise.

**Methods:** Here we report results on the PCR-genotyping of the V1016G mutation in 2530 *Ae. albopictus* specimens from 69 sampling sites in 19 European countries.

**Results:** The mutation was identified in 12 sites from nine countries (with allele frequencies ranging from 1 to 8%), mostly distributed in two geographical clusters. The western cluster includes Mediterranean coastal sites from Italy, France and Malta as well as single sites from both Spain and Switzerland. The eastern cluster includes sites on both sides of the Black Sea in Bulgaria, Turkey and Georgia as well as one site from Romania. These results are consistent

# Thank you for your attention!

# Osório Hugo, National Institute of Health (PT)

# Control of *Aedes albopictus* using the Sterile Insect Technique

## Monitoring and Assessment tools

Hugo Costa Osório

Centre for Vectors and Infectious Diseases

National Institute of Health

# Subject and purpose of the STSM

- **Problem adressed:** *Aedes albopictus* control using SIT
- **Working Group 2:** Conventional & Innovative Control Tools
- **Host Institution:** Benaki Phytopathological Institute (BPI), under coordination of Dr Antonios Michaelakis
- **Date:** 5-19/09/2020 (15 days) and **Target site:** Vravrona, Markopoulo, Greece
- **Main objective:** To test the dispersal capacity and longevity of irradiated and non-irradiated males of *Ae. albopictus* released at the same environmental conditions

# STSM in a glimpse






# Main impact in my scientific work and outputs

- Implementation of SIT under an IVM strategy plan in Portugal
- First Mark-Release-Recapture trial performed in Portugal: Sep-Nov 2022
- Awareness and community involvement based on a communication plan
- SIT pilot supression trial planned for 2023
- **Scientific projects aligned and international collaboration ongoing**

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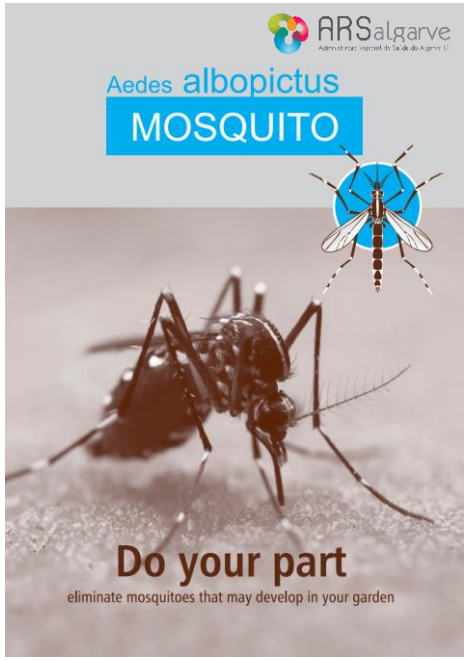
BENAKI PHYTOPATHOLOGICAL INSTITUTE

Instituto_Nacional de Saúde
Doutor Ricardo Jorge






# Kavran Mihaela, University of Novi Sad (RS)

# Kurucz Kornélia, University of Pécs (HU)

UNIVERSITY OF PÉCS
NATIONAL LABORATORY
OF VIROLOGY

# Share the data:
## collaboration of different fields of science

**Kornélia Kurucz**

**Short-Term Scientific Mission in Italy, 2019**

Applied Ecology Research Unit,

Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige

FONDAZIONE EDMUND MACH

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# To become familiar with distribution modeling:

needed for the spatial and temporal spreading analyses of invasive mosquito species

**Database available so far from Hungary**

- Spatial and temporal abundance data of several species
- Over 4 years
- Data on climatic parameters

+

**Analyzed the dynamics & distribution of *Aedes koreicus* in our region**

- Provided the model's suitability for future studies
- **Kurucz et al. 2020** Int. J. Environ. Res. Public Health 2020, 17(8), 2728.

→

**Analyzed pan-European genetic pattern of *Aedes koreicus***

- Revealed general dispersal patterns & necessity of cooperation without borders
- **Kurucz et al. 2022** PLoS ONE 17(8): e0269880.

→ ...

- ✓ Importance of multidisciplinary thinking and data sharing
- ✓ Perfect platform for knowledge transfer and meeting other groups
- ✓ Good basis for further collaborative research
- ✓ To become part of a working network

*To Be Continued...*

# Marini Giovanni, Fondazione Edmund Mach (IT)


# UK mosquitoes: future research

**Giovanni Marini**

**STSM: Imperial College London, 3-14 October 2022**

# STSM details and first impact

- Host: Ilaria Dorigatti (Imperial College London)
- Project: characterizing UK suitability for *Ae. koreicus* and *Ae. japonicus*

**Great opportunity to present my research and to discuss mosquito biology and *Aedes*-borne pathogens with several infectious diseases' experts.**

Funded by the European Union

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# STSM: future research

1. Produce a risk map of UK depicting habitat suitability for *Ae. koreicus* and *Ae. japonicus*.
2. Evaluate the population dynamics of the two species at specific locations.

1+2: We will use the *dynamAedes* R library

Further impact: we will co-supervise together several PhD students, covering also other mosquito species and related pathogens of European interest, such as West Nile virus.

Funded by the European Union

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# Thank you!

**Acknowledgements**

- AIMCOST
- Imperial College London
- Dr. Ilaria Dorigatti

# Rogozi Elton, Institute of Public Health Albania (AL)

FRIEDRICH-LOEFFLER-INSTITUT
FLI
Bundesforschungsinstitut für Tiergesundheit
Federal Research Institute for Animal Health

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INSTITUTI I SHËNDETIT PUBLIK

# "Flavirus surveillance in Aedes mosquitoes from Albania"


**Institute of Public Health, Tirana, Albania** - **Friedrich Loeffler Institute, Greifswald, Germany**

**Elton Rogozi** **Mandy Schäfer**


BG-Sentinel CO2-Lure trap for adult mosquito collection

| | |
|---|---|
| **Adults' Collection** | 1. BG Sentinel + Lure + CO2 traps |
| | 2. Resting Catch – Mechanical aspirator |
| | 3. Human Landing Catches – HLC |

Mosquito processing for Flavivirus detection at FLI

## OBJECTIVE

In order to clarify the occurrence of mosquito-borne Flavivirus in Albania and to identify their potential as a disease causative pathogenicity, we conducted a collection of mosquitoes over a period of three years (2019-2021) along the coastal side and two central regions of Albania.

## MATERIAL AND METHODS

Adult collection was performed via different adult traps, e.g.BG-Sentinel+Lure+$CO_2$ and light traps augmented or Not with $CO_2$.

We monitored 12 stations/locations that were regularly sampled every 2 weeks.

Transport with dry ice to protect potentially Flavivirus-infected mosquitoes from degradation.

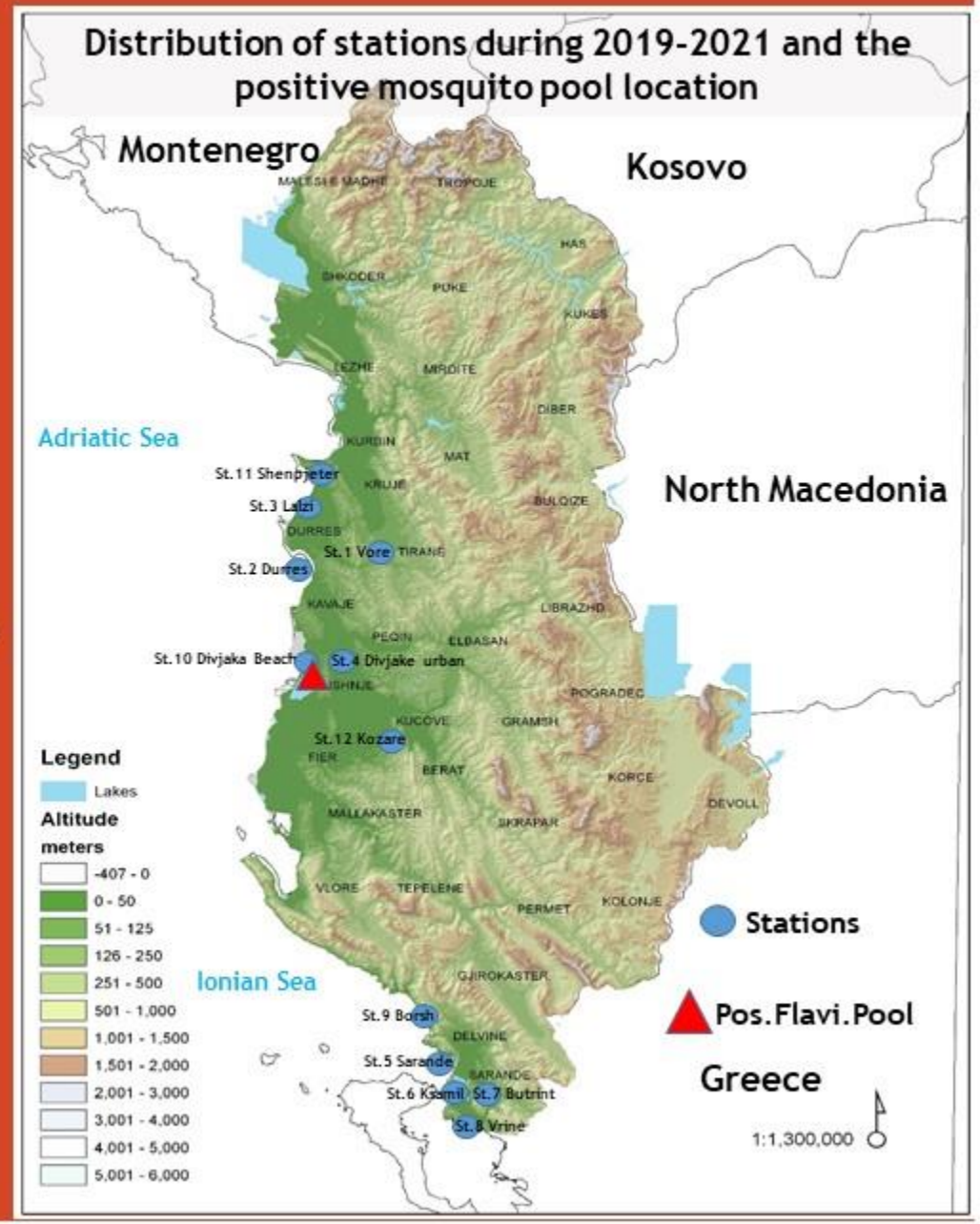
Females and males were placed separately in tubes together with two 3mm metals beads to allow for disruption and lysis of the tissue.

A volume of 500µl of media solution was added to samples containing one individual, and 750µl to pools with 2-27 individuals of adult mosquitoes.

Pan-favivirus RT-qPCR assay was applied to each pool.

### Mosquito Sampling Sites

| | | | |
|---|---|---|---|
| Station 1 | Vore, Tirane | Station 7 | Butrint |
| Station 2 | Durres, urban area | Station 8 | Vrine |
| Station 3 | Lalzi bay | Station 9 | Borsh |
| Station 4 | Divjake urban | Station 10 | Divjake Beach |
| Station 5 | Saranda urban area | Station 11 | Shenpjeter, Durres |
| Station 6 | Ksamil | Station 12 | Kozare, Kucove |



## RESULTS

- 17.222 mosquitoes/1068 pools of 1-27 adult females in total;
- 5 species *Aedes albopictus*, *Aedes caspius*, *Anopheles maculipennis* s.l., *Culex pipiens* and *Culex tritaeniorhynchus*;

We detected a single pool in the beach sandy area of Divjaka with a dense pine forest area, where mosquito abundance and the presence of migratory birds in high.

- The sequence has roughly 82% similarity with Flaviviruses in the NCBI GenBank database.

## Next steps

1. Flavivirus isolation on cell culture.
2. Full genome sequencing of the Flavivirus detected.
3. Possibility of the Flavivirus to cause disease in human and/or animals?

Descriptions | Graphic Summary | Alignments | **Taxonomy**

**Reports** | **Lineage** | **Organism** | **Taxonomy**

*100 sequences selected*

| Organism | Blast Name | Score | Number of Hits | Description |
|---|---|---|---|---|
| Flavivirus | viruses | | 101 | |
| . West Nile virus | viruses | 171 | 79 | West Nile virus hits |
| . Japanese encephalitis virus | viruses | 167 | 2 | Japanese encephalitis virus hits |
| . Banzi virus | viruses | 163 | 2 | Banzi virus hits |
| . Tyuleniy virus | viruses | 143 | 2 | Tyuleniy virus hits |
| . Dengue virus type 2 | viruses | 137 | 16 | Dengue virus type 2 hits |

## CONCLUSIONS

- Our study showed the presence of a new Flavivirus species in adult *Aedes caspius* mosquitoes in coastal areas of Albania.
- Present at sites where the migratory birds are resting.
- Mosquitoes in these area continue to be a serious threat for infections disease transmission in Albania.
- Strategies of vector/mosquito control should be intense and undertaken from different bodies (public and private).

## Future studies

1. Further studies to detect possible pathogen agents on adult mosquitoes in Albania are recommended.
2. Studying the vectorial capacity and competency of invasive *Aedes* ssp. for certain viruses or pathogens.
3. Harmonization of the new approaches for the improvement of the *Aedes albopictus* control in Albania.


## ACKNOWLEDGEMENTS

1. Institute of Public Health Albania: Which had the greatest support on the mosquito collection for 3 years for this research, its interest and continuous support on the importance of these specific studies;
2. AIM-COST: STSM support, possibility to be promoted with the Core European Entomologists network and being involved in specific surveillance and monitoring of the Aedes invasive species of Albania part;
3. Friedrich Loeffler Institute Germany: For the great support to test a large amount of Aedes adult mosquitoes for Flavivirus detection, their continuous support and for future collaborations intended to be realized.

Vanslembrouck Adwine,
Institute of Tropical Medicine,
Antwerp, (BE)

# Carry-over effects of different larval competition treatments on arboviral vector competence

Adwine Vanslembrouck, Anna Heitmann, Stephanie Jansen, Renke Lühken, Jonas Schmidt-Chanasit, Ruth Müller

INSTITUTE OF TROPICAL MEDICINE ANTWERP

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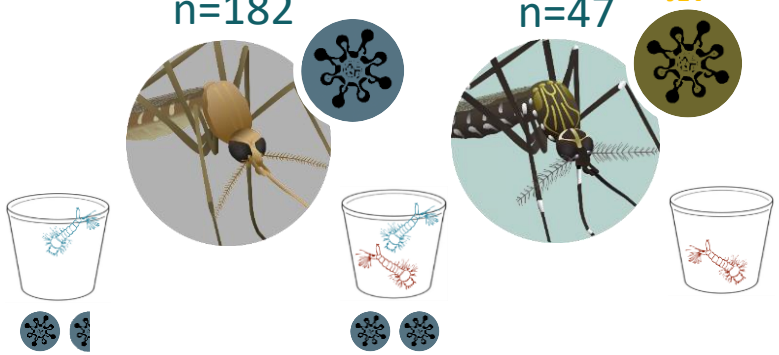
## Intra- and interspecific larval competition experiments


## Vector Competence studies @ BNITM



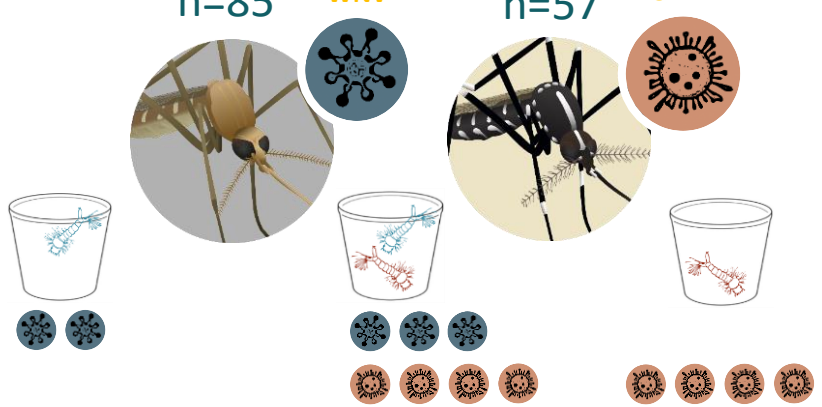
*Culex pipiens* n=182 WNV

*Aedes japonicus* n=47 JEV



*Culex pipiens* n=85 WNV

*Aedes albopictus* n=57 CHIKV



Higher infection rate and body titer per mosquito for interspecific combinations

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## Impact of STSM on my scientific growth and career

- Training in Vector Competence studies in BSL-3
- Building a scientific network at BNITM
- International collaborative perspectives: joint paper "Carry-over effects of larval competition between the mosquito species *Aedes albopictus*, *Aedes japonicus* and *Culex pipiens* on their arboviral vector competence" → chapter PhD
- Results show differential infection rate in response to competition treatment
- Scientific impact: first time tested


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