



Surveillance of invasive and native mosquito vectors
and pathogens they transmit in Montenegro



**LOVCEN
FIRST ANNUAL MEETING MAY 13-15, 2015**

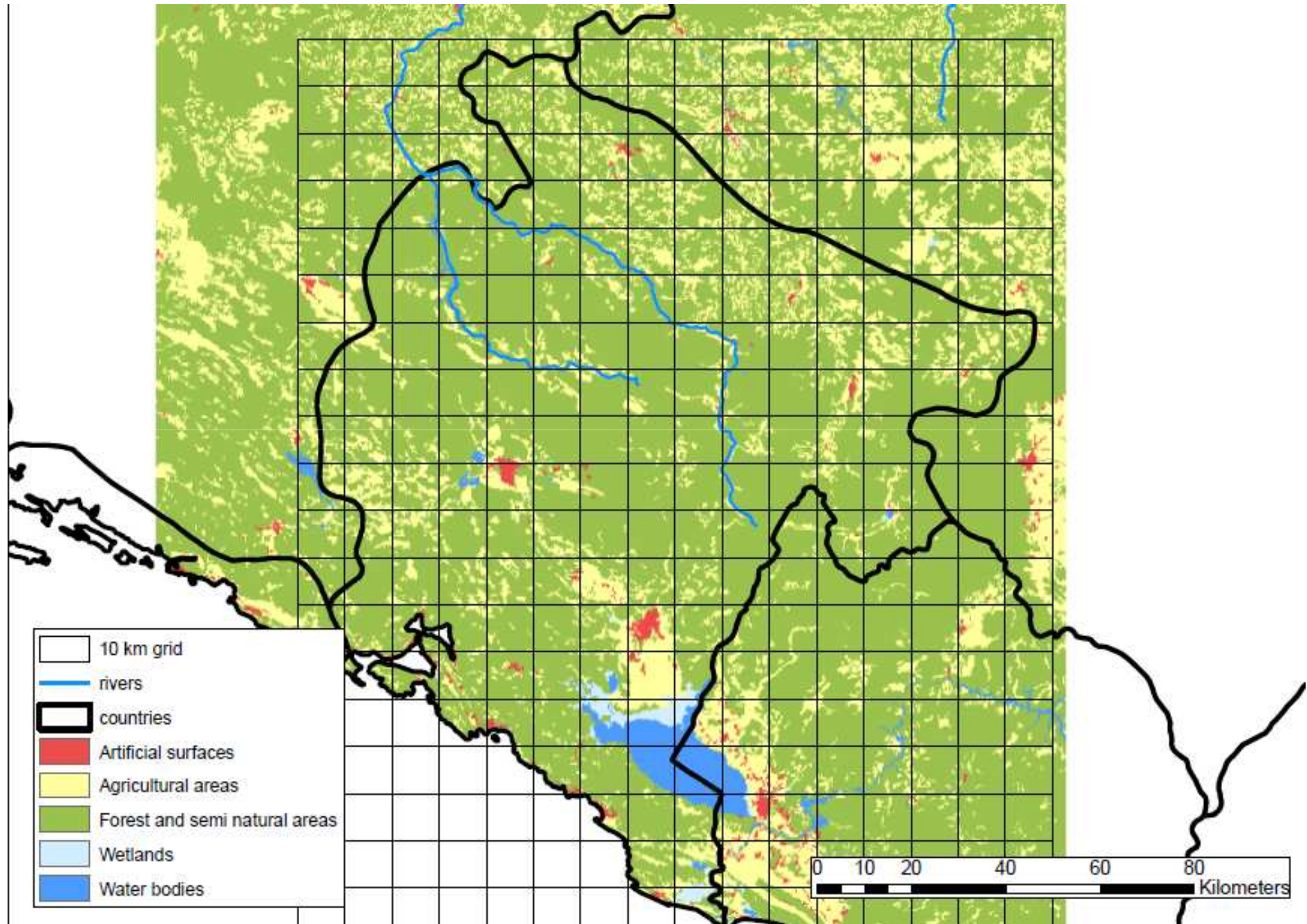
**WP1 Collaborative research on
native/invasive mosquitoes and pathogens
they transmit in Montenegro and
development of non-chemical control
measures**

**Romeo Bellini
CENTRO AGRICOLTURA AMBIENTE "G.NICOLI"
Medical and Veterinary Entomology Department
CREVALCORE (BO), ITALY**

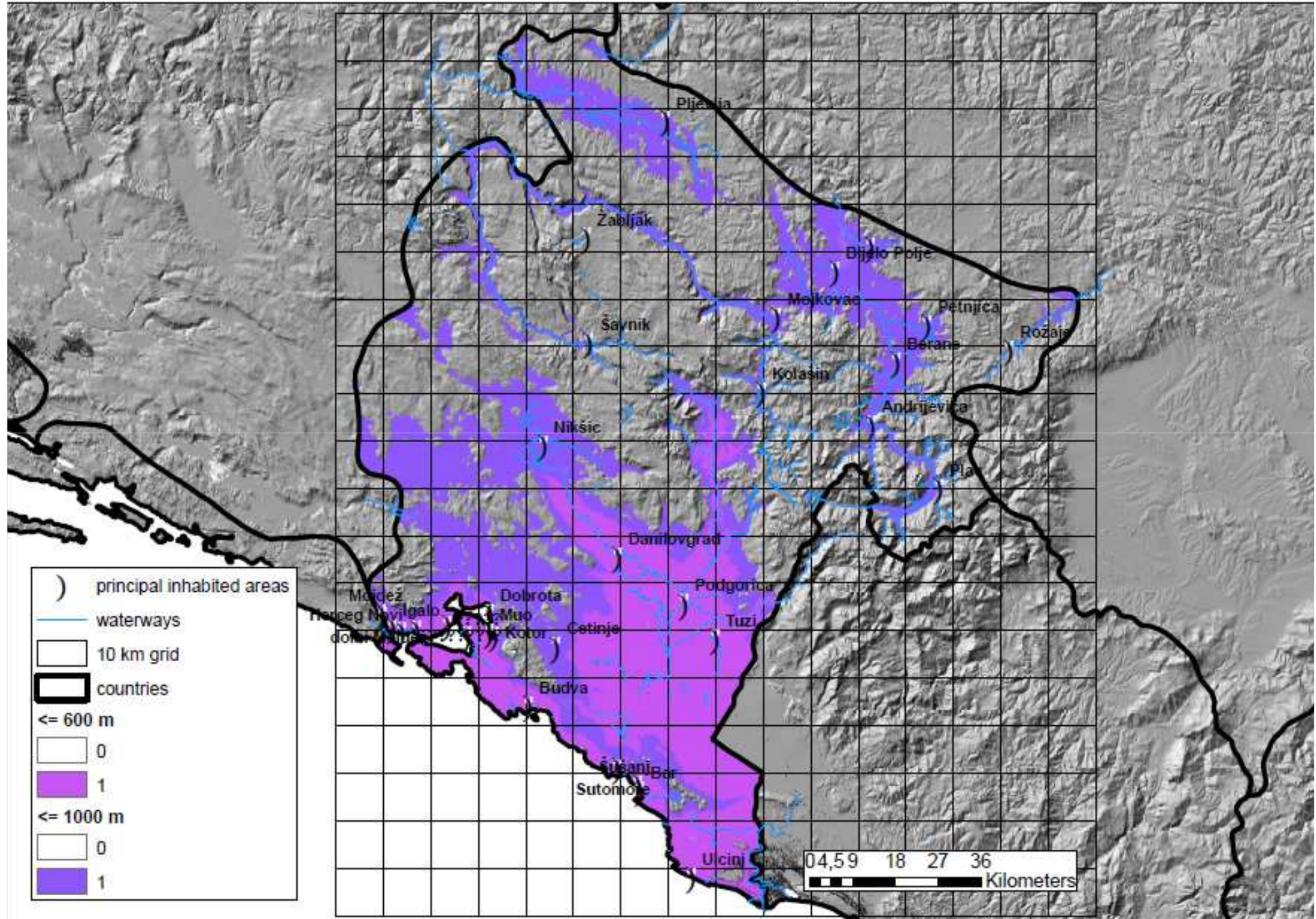
WP1 Collaborative research on native/invasive mosquitoes and pathogens they transmit in Montenegro and development of non-chemical control measures

- **How to start a sustainable surveillance system on mosquito and MBD in Montenegro ?**
- **Identify the possible points of entry**
- **How to develop the risk assessment ?**
- **Which the impact of mosquitoes on the life quality ?**
- **Which the geographic distribution of the noxiousness ?**
- **How to start the cost-benefit analysis including both public health and tourism ?**
- **How to be proactive on new mosquito control methods with low/no environmental impact ?**

GRID ASSISTING THE PLANNING OF MOSQUITO SURVEILLANCE MNE



GRID ASSISTING THE PLANNING OF MOSQUITO SURVEILLANCE MNE





INFRAVEC
FINAL CONSORTIUM & DISSEMINATION MEETING
Imperial College London
London, 27th – 28th February 2014



Feb 2012

ID: 0012

MONTENEGRO

“Testing the shipment methods and the international regulation for sterile mosquitoes delivery”

INFRAVEC

ID: 0012

MONTENEGRO

- **Understanding of the regulation issues, safety measures and documentary dossier, dealing with the shipment of *Ae. albopictus* sterile males produced at CAA Italy to Montenegro**
- **Verifying the timing of fast delivering, system of containment of the sterile males and their quality at the arrival site**

CRNA GORA
VLADA CRNE GORE
AGENCIJA ZA ZAŠTITU ŽIVOTNE SREDINE
02 Broj UPI-833/10
Podgorica, 16.09.2013. godine
JL

Na osnovu člana 81 stav 3 Zakona o zaštiti prirode („Sl.list CG“, br.51/08, 21/09, 40/11), člana 22 stav 3 Zakona o spoljnoj trgovini („Sl.list RCG“, br.28/04, 37/07), člana 9 Odluke o kontrolnoj listi za izvoz, uvoz i tranzit robe („Sl.list CG“, br. 10/11, 15/12) i člana 196 Zakona o opštem upravnom postupku („Sl.list RCG“, br. 60/03, „Sl.list CG“, br. 32/11), a rješavajući po zahtjevu Biotehničkog fakulteta – Univerzitet Crne Gore iz Podgorice, broj: 02-UPI-833/1 od 08.07.2013. godine, Agencija za zaštitu životne sredine *donosi*

RJEŠENJE

I **DOZVOLJAVA SE** Biotehničkom fakultetu – Univerzitet Crne Gore uvoz 10 000 (slovima: deset hiljada) adulta sterilnih mužjaka vrste komarca *Stegomyia albopicta* i njihov unos u ekosistem na lokaciji poluostrvo Luštica u naučno obrazovne svrhe.

Uvoz materijala predmetne životinjske vrste će se vršiti na graničnom prelazu Aerodrom Podgorica.

II Dozvola se izdaje na period od jedne godine od datuma izdavanja.

Obrazloženje

Biotehnički fakultet – Univerzitet Crne Gore iz Podgorice podnio je ovom organu zahtjev br.02-UPI-833/1 od 08.07.2013. godine za izdavanje dozvole za uvoz 10 000 adulta sterilnih mužjaka vrste komarca *Stegomyia albopicta* u naučno obrazovne svrhe.

Nakon razmatranja predmetnog zahtjeva ovaj organ je našao da je uvoz i tretman jedinki navedene životinjske vrste, koja **nije zaštićena** Rješenjem o stavljanju pod zaštitu pojedinih biljnih i životinjskih vrsta („Sl.list RCG“, br. 76/06), **moгуće vršiti** u naučno istraživačke svrhe, u količinama i na način kako je navedeno u Zahtjevu, čime su se stekli uslovi za izdavanje dozvole podnosiocu zahtjeva.

.....omissis
ADMINISTRATIVE DECISION

WE ALLOWED Biotechnical faculty – University of Montenegro to import 10,000 (letters: ten thousand) sterile adult males mosquitoes species *Stegomyia albopicta* and their introduction in ecosystem on location peninsula Lustica for scientific and educational purposes.
Import of material will be on border cross Airport Podgorica.
This permit is valid one year from the date of issue.

16/09/2013

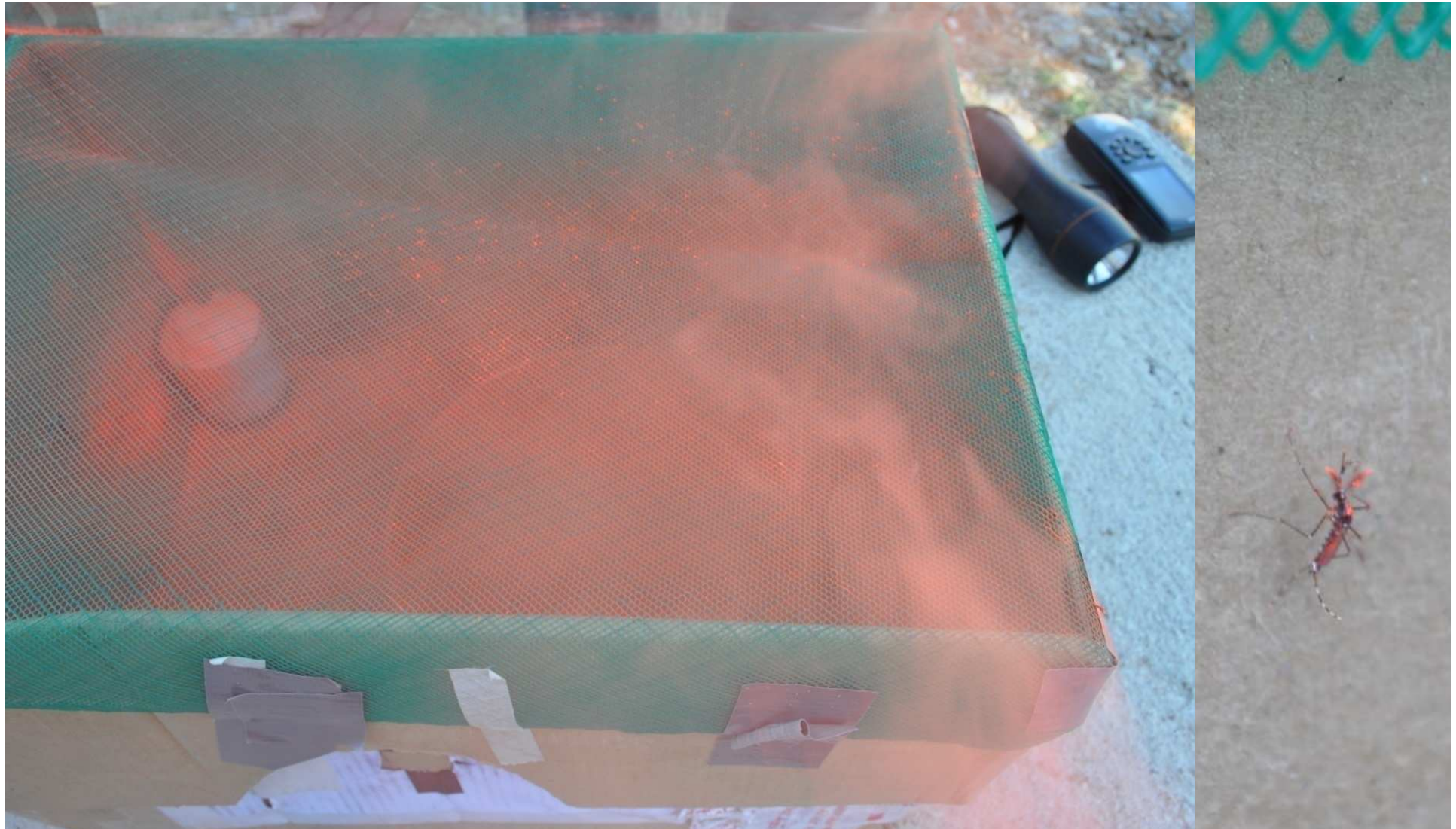
INFRAVEC

ID: 0012

MONTENEGRO

- **5,000 eggs August 2013 (DHL taken the 26/08/2013, 5.48PM – delivered the 28/08/2013, 3.43PM in 46 hours)**
- **7,500 L4-pupae provided by CAA for mark-recapture study September (DHL taken the 02/09/2013, 4.48PM – delivered the 04/09/2013, 11.29AM in 43 hours)**

Sterile males marking with fluorescent dust



WP1 TOPIC
IN SITU TRAINING IN RESEARCH ACTIVITIES -
IMPLEMENTATION OF MOSQUITO SURVEILLANCE AND
SIT/NON-CHEMICAL CONTROL MEASURES IN
MONTENEGRO - 15th-20th August 2014

- **to develop surveillance methods of MV/MBD;**
- **to develop possible environmentally friendly mosquito control measures including Sterile Insect Technique;**
- **to plan possible research activities;**
- **to provide specific practical training to researchers from BTF.**

TARGET IMS SPECIES

ECDC GUIDELINES 2012

- ***Aedes albopictus***
- Others IMS (***Ae. aegypti***, *Ae. atropalpus*, *Ae. japonicus*, *Ae. koreicus*, *Ae. triseriatus* and *Ae. cretinus*)

ALBO MANAGEMENT PLAN COMPONENTS

- Standardized quantitative monitoring (in some areas)
- Public health risk assessment
- Community participation
- Standard control measures in public areas
- Standard control measures in private areas
- Emergence control measures
- Pilot door-to-door control measures in private areas
- Efficacy & quality control methods
- Resistance prevention

ALBO MANAGEMENT PLAN

Standardized quantitative monitoring aims

- to estimate the vector density level during the favorable season;
- to estimate the sanitary risk for VBD;
- to evaluate the impact of vector control measures on the vector density;
- to evaluate the possible long term population dynamic fluctuations.

ALBO MANAGEMENT PLAN

Standardized quantitative monitoring actions

- prefix the precision level to be achieved in the estimation of the vector density and its spatial significance (e.g. $d=0.25$);
- define the tool to be adopted (e.g. ovitraps type);
- define the monitoring period;
- define the grid on the urban areas under monitoring considering the number of ovitraps to be positioned;
- first ovitrap positioning must be performed by highly skilled technicians;
- SOP and formation for the field operators managing ovitraps;
- SOP and formation for the operators counting eggs;
- quality control procedure to be applied regularly;
- data management, dissemination, processing (www.zanzaratigreonline.it).

ALBO MANAGEMENT PLAN

Public health risk assessment

From the R_0 equation

$$R_0 = \frac{ma^* (a S_m V S_v p^i)}{(- \log_e p)}$$

(Fine 1981, Reisen 1989)

Parameter	Label	Value	Reference
Multifeeding/gonotrophic cycle	mF	1.20	Hawley 1988
Host Feeding Pattern	AI	0.86-0.96	Valerio et al. 2010
Gonotrophic cycle	GC	4 - 11 days	Calculated in function of temperature by means the model of Focks et al. 1993.
Vector competence	Sm	Chik.: 24 – 80%	Vazeille et al. 2007 Talbalaghi et al. 2010 Mitchell 1991
Viremia	V	6 days	Peters and Dalrymple 1990 Boelle et al. 2008
Females daily survival rate	p	0.90	Hawley 1988 Willis and Nasci 1994 Almeida et al. 2005
Extrinsic incubation period	i	EIP=0.71GC	Dubrulle et al. 2009 Hawley 1988
Population susceptibility to Dengue and CHIKV	S_V	1	Moro et al. 2010
Vectorial capacity correction factor	X_V	0.101	Calculated
Bites per Egg Rate	B	PDS: 0.033 ± 0.015 HLC: 0.042 ± 0.021 NBC: 0.027 ± 0.028	Calculated

ALBO MANAGEMENT PLAN

Public health risk assessment

Pearson product moment correlations (R)

Sampling method	Mean number of eggs/week/ovitrap		
	Previous week	Sampling week	Following week
PDS – Pupae per hectare	0.1703	0.3396	0.8622**
NBC – (No. bites received the day before the adult sampling)	0.6279	0.7243*	0.6318
HLC – (No. biting females caught in 15 min)	0.7092*	0.8604**	0.7013*

Public health risk assessment

Epidemiological thresholds based on the N° eggs also

N.eggs/ 14 days	Chik E1-Ala226Val	Chik	Dengue
< 250	$R_0 < 1$	$R_0 < 1$	$R_0 < 1$
250-450	$1 < R_0 < 2$	$R_0 < 1$	$R_0 < 1$
451-750	$2 < R_0 < 3$	$1 < R_0 < 2$	$R_0 < 1$
751-1000	$R_0 > 3$	$1 < R_0 < 2$	$1 < R_0 < 2$
1001-1500	$R_0 > 3$	$2 < R_0 < 3$	$1 < R_0 < 2$
>1501	$R_0 > 3$	$2 < R_0 < 3$	$2 < R_0 < 3$

ALBO MANAGEMENT PLAN

Community participation

- Education in school;
- Citizen information (TV, radio, news, web);
- Mayor ordinance;
- Distribution of domestic insecticides;
- Non profit organizations involvement;
- Efficacy evaluation.

ALBO MANAGEMENT PLAN

Standard control measures in public areas

- census and mapping of public road drains;
- larval control in road drains: (e.g. 5 treatments per season with IGR);
- adult control in specific sites following specific need analysis (e.g. schools, urban green areas).

ALBO MANAGEMENT PLAN

Standard control measures in private areas

- Mayor ordinance;
- Domestic water containers elimination;
- Larval control in permanent breeding sites;
- Mosquito proof measures adoption (e.g. covering of container).

ALBO MANAGEMENT PLAN

Emergency control measures in case of DEN & CHIK imported case detection (suspected or confirmed)

- based on the vector density observed;
- prompt activation of vector control operations (< 24 hrs);
- identification of the areas to be submitted to vector control (100 m ray for singular case, 300 m ray for cluster);
- in public areas: three consecutive adult control treatments during the night + larval control in road drains;
- in private areas: adult + larval control door-to-door during the day;
- suspend the operation in case the suspected case is not lab confirmed.

ALBO MANAGEMENT PLAN

Pilot door-to-door control measures in private areas

- Five larval control treatments in the public road drains with IGR (Diflubenzuron) (minimum threshold of treated road drains 95%);
- Five door-to-door interventions (larval control, source removal and citizen direct education) (minimum threshold of properties inspected 95%);
- Identification and reporting to the authorities of the non-collaborative citizen;
- Quality control operations on mosquito control activities;
- Intensive monitoring with ovitraps;
- Introduction of Copepods in large water containers;
- Sterile males releases in specific sites;
- Adulticide treatments only in case of real needs;
- Info contact green point (web, phone);
- Socio-economic and environmental impact evaluation of management plan.

ALBO MANAGEMENT PLAN

- **Quality control methods: larval control in public road drains**
- Census and mapping of the public road drains;
- In case the PCO mark the road drains, inspects the rate of marking in the 3-5% of the road drains, or GPS control;
- 0.8% of the total road drains censed should be sampled following each treatment;
- In small localities a minimum number of 20 road drains will be sampled in each treatment;
- Sampling will be conducted in the pre-defined time window (e.g. 10-20 days post-treatment for Diflubenzuron) with a water net and number of larvae/pupae recorded;
- the PCO must regularly report on the treated roads;
- Weekly reporting to the Municipality.

ALBO MANAGEMENT PLAN

Resistance prevention

- Maintain the record of insecticide used in each localities & year;
- Each year consider some localities to be checked for albo population sensitivity;
- Collect about 2,000 eggs in each locality;
- Conduct standard bioassays for LD calculation in parallel on field and reference lab larvae.

ALBO MANAGEMENT PLAN

ANNEX LIST

- SOP for ovitraps field management;
- SOP for eggs counting;
- Quality control procedure for the *Aedes albopictus* monitoring;
- Mayor ordinance scheme;
- Protocol for emergence vector control operations in case of Den & Chik cases detection;
- Quality control procedure for larval treatments efficacy in road drains;
- Protocol for bioassays on insecticide sensibility;
- Public tender for PCO.

AEGYPTI MANAGEMENT PLAN

- Immediate formal communication to the Public Health authorities and involved Municipalities;
- extended surveillance to define the colonized area;
- immediate application of intensive capillary control measures aimed to the maximum reduction of population density;
- Public information;
- Local and Regional authorities involvement;
- Quality control methods.

AEGYPTI MANAGEMENT PLAN

Immediate formal communication to the Public Health authorities and Municipalities

- immediate emanation of Public Health regulation to support extraordinary measures;
- organization of a crisis unit including representatives of stakeholders and authorities;
- obtain a mandate from the court allowing to entry in private properties for public health emergence.

AEGYPTI MANAGEMENT PLAN

Enlarge the surveillance to define the colonized area

- prepare a series of maps of different scales of the area including the site of first detection (P. of E.);
- position ovitraps (and BG traps) at a progressive distance from the site of detection considering the more favourable ecological condition;
- as a first step 20-30 ovitraps should be placed in an area of about 1 kmsq, and enlarged in case of new positivity's;
- analyse together with local authorities/stakeholders the possible way of introduction of the species and inform other entities of the risk;
- analyse together with local authorities the possible pattern of passive dispersion and inform other entities of the risk (e.g. customers of positive used tyres company).

AEGYPTI MANAGEMENT PLAN

Immediate application of intensive capillary control measures aimed to the maximum reduction (possible elimination) of initial population

- Larval control treatments in the public road drains with IGR (Diflubenzuron) (minimum threshold of treated road drains 100%);
- door-to-door interventions (larval control, source removal and citizen direct education) (minimum threshold of properties inspected 100%);
- Identification and reporting to the authorities of the non-collaborative citizen;
- Quality control operations on mosquito control activities;
- Intensive monitoring with ovitraps (one ovitrap per ha);
- Adulticide treatments in case of detectable presence of adults;
- Info contact green point (web, phone).

AEGYPTI MANAGEMENT PLAN

Public information

- immediate public information using local media requiring active participation (TV, radio, newspapers,...);
- continue the information campaign explaining the results achieved;
- Stress the need of community involvement to face the sanitary risk.

AEGYPTI MANAGEMENT PLAN

Local and Regional authorities involvement

- Stimulate the organization of a crisis unit including all entities such as Public Health Regional and Local, Epidemiology Services, Municipalities, port and airport authorities, expert entomologists;
- The crisis unit must define responsibilities, money allocation, coordination of the activities, adapt the strategy to the observed evidences.

AEGYPTI MANAGEMENT PLAN

Quality control methods

- the crisis unit must commission an independent body the quality control activities to check the efficacy of vector control;
- 10% of the total road drains in the treated area should be sampled following each treatment;
- sampling will be conducted with a water net and number of larvae/pupae recorded and kept for species determination;
- the PCO must regularly report on the treated roads;
- weekly reporting on the findings to the crisis unit.

ANNEX LIST TO THE AEGYPTI MANAGEMENT PLAN

- SOP for species discrimination;
- Mayor ordinance scheme;
- Protocol for emergence vector control operations in case of Den & Chik cases detection;
- Quality control procedure for larval treatments efficacy in public & private areas;
- Protocol for bioassays on insecticide sensibility.

OTHERS IMS MANAGEMENT PLAN

(Ae. atropalpus, Ae. japonicus, Ae. koreicus, Ae. triseriatus and Ae. cretinus)

- Extended surveillance to define the colonized area
- Application of control measures
- Public information
- Local and Regional authorities involvement
- Quality control methods

OTHERS IMS MANAGEMENT PLAN

Extend the surveillance to define the colonized area

- prepare a series of maps of different scales of the area including the site of first detection (P. of E.);
- position ovitraps (and other traps depending from the species) at a progressive distance from the site of detection considering the more favourable ecological condition;
- as a first step 10-20 ovitraps should be placed in an area of about 1 kmsq, and enlarged in case of new positivity's;
- analyse together with local authorities/stakeholders the possible way of introduction of the species and inform other entities of the risk;
- analyse together with local authorities the possible pattern of passive dispersion and inform other entities of the risk (e.g. customers of positive used tyres company).

OTHERS IMS MANAGEMENT PLAN

Application of control measures

- Larval control treatments in the public road drains with IGR (Diflubenzuron);
- door-to-door interventions (larval control, source removal and citizen direct education);
- Identification and reporting to the authorities of the non-collaborative citizen;
- Quality control operations on mosquito control activities;
- Monitoring with ovitraps (one ovitrap per 5ha);
- Adulticide treatments in case of detectable presence of adults;
- Info contact green point (web, phone).

OTHERS IMS MANAGEMENT PLAN

Public information

- immediate public information using local media requiring active participation (TV, radio, newspapers,...);
- continue the information campaign explaining the results achieved;
- Stress the need of community involvement to face the spread of the species.

OTHERS IMS MANAGEMENT PLAN

Local and Regional authorities involvement

- Inform responsible entities such as Public Health Regional and Local, Epidemiology Services, Municipalities, port and airport authorities, expert entomologists;
- Depending from the national context define responsibilities, money allocation, coordination of the activities, adapt the strategy to the observed evidences.

OTHERS IMS MANAGEMENT PLAN

Quality control methods

- quality control activities aiming to check the efficacy of vector control should be conducted by an independent body;
- 1% of the total road drains in the treated area should be sampled following each treatment;
- sampling will be conducted with a water net and number of larvae/pupae recorded and kept for species determination;
- the PCO must regularly report on the treated roads;
- Regular reporting on the findings to the authorities.

ANNEX LIST TO THE OTHERS IMS MANAGEMENT PLAN

- SOP for ovitraps field management;
- SOP for eggs counting;
- Mayor ordinance scheme;
- Quality control procedure for larval treatments efficacy in road drains;
- Protocol for bioassays on insecticide sensibility;
- Public tender for PCO (?).

IAEA Regional Project

Integrating the use of SIT as an area wide component of the vector management programmes against Aedes invasive mosquitoes in the European Region

Participating countries / institutions

Albania	Institute of Public Health, Tirana (Enkeleida Dikolli - keladikolli@yahoo.com)	South East European Center for Infectious Diseases Surveillance and Control Silvia Bino - silviabino@gmail.com)
Bosnia and Herzegovina	Ministry of Health , Sarajevo, , (Aida Pilav, aida.pilav@fmoh.gov.ba	Institute of public Health, Banja Luka, Nina Rodic Vukmir , nina.rodic@gmail.com
Bulgaria	National Center of Infectious and Parasitic Diseases, Sofia (Ognyan Mikov - mikov@ncipd.org)	Regional Environmental Centre, Sofia (Vesselin Drobenov - vdrobenov@rec.org)
Croatia	University of Osijek, Dept. of Biology, Osijek (Enrih Merdic - enrih@biologija.unios.hr)	National Institute of Public Health, Zagreb, Iva Pem Novosel, iva.pem- novosel@hzjz.hr
Cyprus	Cyprus Center for European and International Affairs, Nicosia (Stephanie Christou - christou.st@unic.ac.cy)	
France	Institut de recherche pour le développement, Marseille (Didier Fontenille - didier.fontenille@ird.fr)	
Greece	University of Thessaly, School of Agricultural Sciences, Laboratory of Entomology and Agricultural Zoology, Volos (Nikolaos Papadopoulos - nikopap@uth.gr)	Benaki Phytopathological Institute, Kifisia, Athens (Antonios Michaelakis - a.michaelakis@bpi.gr)

Participating countries / institutions

Italy	Centro Agricoltura Ambiente "G.Nicoli", Crevalcore - IAEA Collaborating Centre (Romeo Bellini - rbellini@caa.it)	ENEA, Rome (Maurizio Calvitti - maurizio.calvitti@enea.it)
Kosovo	National Institute of Public Health, Naser Ramadani , naseri0000@yahoo.co.uk	
Macedonia	Centre for Public Health, Skopje (Lazarevska Liljana - llazar@cjzsk.org.mk)	
Moldova	National Center of Public Health, Ministry of Health, Chisinau, Stela Gheorghita gheorghitastela11@gmail.com	
Montenegro	University of Montenegro, Biotechnical Faculty, Podgorica (Igor Pajovic - pajovicigor@yahoo.com)	

Participating countries / institutions

Portugal	Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisbon (Carla Sousa - CaSousa@ihmt.unl.pt)	Center for Vectors and infectious Diseases Research of the National Institute of Health, Lisbon (Maria João Alves - m.joao.alves@insa.min-saude.pt)
	Antonio Miguel Franquinho Aguiar Antonio.aguiar@sra.pt Laboratorio Agricola da Madeira	
Romania	National Institute for Research and Development in Microbiology and Immunology Cantacuzino, Bucarest (Gabriela Nicolescu - gabrielamarianicolescu@yahoo.co.uk)	
Serbia	University of Novi Sad, Faculty of Agriculture, Novi Sad (Dusan Petric - dusanp@polj.ns.ac.yu)	
Spain	Tragsa, Valencia (Carles Tur Lahiguera - ctur@tragsa.es)	Consell Comarcal del Baix Llobregat, Sant Feliu de Llobregat, Barcelona (Roger Eritja - reritja@elbaixllobregat.cat)
Turkey	Hacettepe University, Faculty of Science, Ankara (Bulent Alten - kaynas@hacettepe.edu.tr)	Istanbul University, Faculty of Veterinary Medicine, Istanbul (Kerem Oter - oter@istanbul.edu.tr)