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SCIENCE FOR A SUSTAINABLE DEVELOPMENT

MOSQUITO VECTORS OF DISEASE: SPATIAL BIODIVERSITY, DRIVERS OF CHANGE AND RISK

"MODIRISK"

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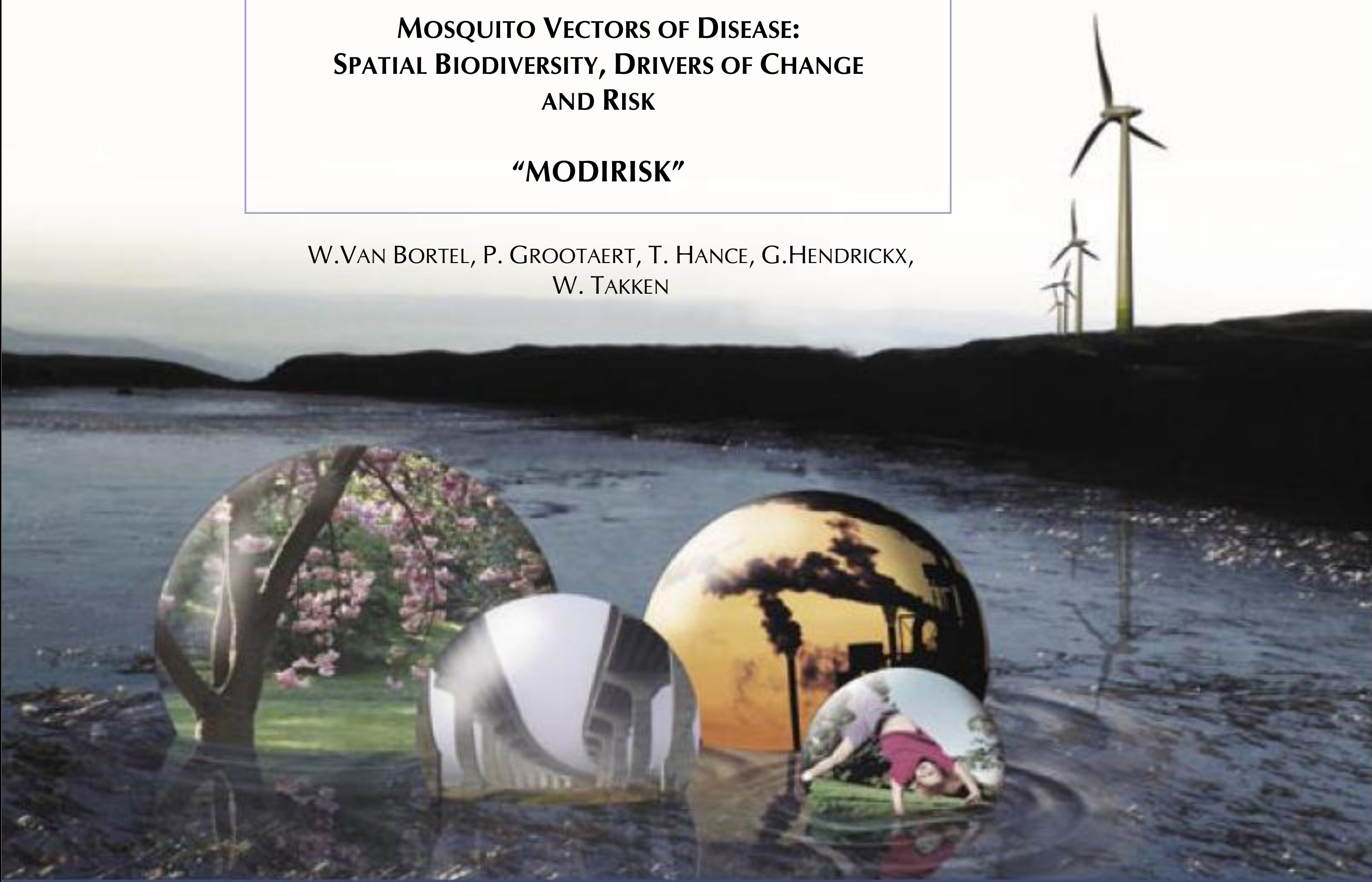

ENERGY
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SCIENCE FOR A SUSTAINABLE DEVELOPMENT
(SSD)

Biodiversity

FINAL REPORT PHASE 1
SUMMARY

MOSQUITO VECTORS OF DISEASE: SPATIAL BIODIVERSITY, DRIVERS OF CHANGE AND RISK

"MODIRISK"

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Ongoing eco-climatic changes create suitable conditions for the (re)emergence of vector-borne diseases in Europe. Of these, mosquito-borne diseases are prime candidates (e.g. recent West Nile Fever events, records of introduction/spread of exotic *Aedes albopictus* in Europe, outbreaks of Chikungunya in Italy and Dengue in Europe overseas territories). Knowledge of the taxonomic and functional biodiversity of both endemic and invading vector mosquito species as well as the factors driving change, is missing in Belgium. Acquiring this knowledge is an essential step towards understanding current risk and preparing for future treads. Therefore the objectives of the project MODIRISK are (1) to inventory endemic and invading mosquito species in Belgium considering environmental and taxonomic elements of biodiversity, (2) to assess the population dynamics of endemic and invasive mosquito species and their interrelationship (3) to model mosquito biodiversity distribution at a one km resolution in the Benelux, and (4) to disseminate project outputs to the scientific community, end users and the general public. During the first phase (years 2007-2008), the project focussed on the inventory activities, the setting–up laboratory experiments for studying life history traits of *Culex pipiens* in relation to temperature and the first selection of spatial distribution models based on the field results.

The cross-sectional field survey was conducted in 2007 and 2008 by use of a network of CO_2-baited Mosquito Magnet Liberty Plus traps throughout Belgium in three key habitats. These habitats (urban, agriculture and nature) were selected based on the Corine database. Twenty seven traps operated simultaneously (9 by team). Each trap operated seven days on one study site after which it was placed on the next study site. Field work was done on Monday, Tuesday, and Wednesday: each day three traps were emptied and replaced. The remaining days were used for the organisation of the field work and the morphological identification of the collected mosquitoes. Project staff (2 persons ITM, 2 persons RBINS, 1 person UCL, 1 person WUR) was trained in state of the art taxonomic identification using morphological techniques. Training was provided by an expert in morphology of European mosquitoes based on reference collection from the expert and from field collected mosquitoes. To assure quality of morphological identification the expert checked the mosquitoes identified by the team members of the MODIRISK project.

For the inventory 936 sites were randomly selected of which 97% was sampled. This success was only possible because three teams from three partner institutes contributed to the inventory and because of the tools developed by MODIRISK to facilitate the field work. These tools included a website, a palm-to-web tool and a database and they serve now as an example to implement a cluster of spatial mosquito sampling and modelling projects in several European countries as part of the IAP program of ESA (European Space Agency).

After two years of intensive inventory 23 Culicidae species belonging to 6 genera were found. The number of species will certainly increase since some species complexes still need to be identified by molecular means. The number of caught species is close to the expected number of species (about 27 species) possibly present in Belgium. The most species rich genus in Belgium is *Ochlerotatus* whereas *Coquillettidia* is only represented by 1 species. The most abundant species was *Culex pipiens* which is found in a large variety of breeding sites. *Coquillettidia richiardii* was the second most prevalent mosquito. This is however due to one study site, a nature reserve in the harbour of Antwerp, where more than 3700 specimens of this species were collected. The species was caught in only 38 study sites. Interestingly is the general occurrence of *Anopheles* species, mainly *Anopheles claviger and Anopheles plumbeus.* The latter is becoming a nuisance species in The Netherlands and Belgium.

Additionally, sites in import risk areas were sampled to evaluate the presence exotic mosquito species in Belgium. Two exotic species were found, *Ochlerotatus japonicus japonicus* in the province of Namur and *Oc. (Finlaya) sp.* in the province of Limburg.

The latter could not be identified to species level and a comparative morphological study will be done at the Smithsonian (Washington DC), whilst Rutgers University (New Brunswick) will be visited to compare molecular data on this species.
A molecular identification method based on the DNA barcoding approach is under development and will facilitate the identification of species complexes and species with overlapping morphological characters and will become an essential tool for studying the taxonomic biodiversity of Culicidae. Preliminary analysis of the DNA barcoding region (COI mtDNA) base on a neighbour joining phylogenetic tree reconstruction method confirms the utility of the COI region for species identification.

All partners involved in the morphological identification started their own reference collection. Therefore 1-5 correctly identified specimens per species were pinned and stored in insect boxes. The remaining specimens were stored in small tubes for subsequent molecular identification. At the RBINS all un-identified already mounted mosquito-specimens from the Belgian collection of the Entomology Department were identified and added to the identified collection. About 4000 specimen were screened during winter 2007-2008 and summer 2008 at the RBINS. At the end all these records and many other individual records and records from other projects at the RBINS will be added to a newly established database CULIBEL (all Belgian Culicidae records). Next, this database will be integrated into the Belgian Biodiversity Platform and will be kept updated by RBINS.

A laboratory colony of *Culex pipiens* was set-up at Université Catholique de Louvain (UCL) to enable the study of the impact of temperature on life history traits of the most wide spread mosquito species from Belgium. Different protocols have been tested to maximize the productivity of the colony of *Cx pipiens*. Two membrane feeder systems (parafilm and skin membrane) and a cotton-stick feeding were tested to feed the females. The latter system showed a higher percentage of engorged females, hence this system was adopted. Three cages are maintained for adult reproduction. To avoid inbreeding, larvae from the field were collected mid May 2008. The adults were morphologically identified and only *Cx pipiens* individuals were released into the three reproductive cages.

The laboratory experiments with Cx pipiens showed that males emerged 1.2 to 5.4 days before the females and that the larval development time increase with lower temperatures. Although not excessive, larvae reared at low temperatures (T15, T11) gave bigger adults than the larvae reared at high temperatures (T28, T20). Furthermore the results show that temperature influence the time required to obtain copulation in *Cx pipiens*. The parameter temperature has a great influence on development and mating activity of *Cx pipiens* and the results of these laboratory tests will be included in the models. The experiments will be repeated by two additional temperatures (T35 and T40).

During phase 1 of the project a spatial data archive of low resolution remote sensing data was developed and eco-climatic zones were identified using an unsupervised k-means clustering. In the first phase, the objective was to determine whether the data extracted from the MODIS data-series were useful for the prediction of mosquito distribution. The distribution models were tested on two species namely *Anopheles claviger* and *Aedes cinereus*. For both species, a training sample was selected, divided over both the presence and absence category. The explanatory variables, composed of 28 data layers (i.e. first three amplitudes and phases of the Fourier transforms, and mean values for day-time land surface temperature (LST), night-time LST, NDVI and EVI), were standardised prior to the statistical modelling to facilitate model output interpretation. The stepwise regression procedure was successful in ruling out a considerable number of explanatory variables. This did however not decrease the predictive value of the models. For *An. claviger* 64% of the absences were correctly classified, and 82% of the presences were correct. For *Ae. cinereus* 94% of the observed absences was correctly classified, as compared to 87% of the observed presences. *An. claviger* individuals have a larger probability to occur in areas with relatively lower vegetation abundance and higher bi-annual vegetation variation. *Aedes cinereus* prefers higher amounts of vegetation, and smaller variations in vegetation amount throughout the year. It was thus proven that different mosquito species show a distinct distribution over the study area, and that eco-climatic variables are paramount to explain a part of this variation. In a second phase of the project models will be extended to other species. Additional field surveys will be conducted in the second phase of the project in Belgium and in The Netherlands to validate and further fine tune the produced models. The model outputs will enable us to understand the factors (mainly eco-climatic, but also human driven such as land use, urbanisation) determining observed distribution patterns. When included in a GIS model, they will also enable to highlight Culicidae biodiversity hotspots which are of prime importance when addressing the issue of emergence of diseases.

The inventory done in the framework of the MODIRISK project is based on a random (statistical) approach that is designed for model building. This is unique in Europe (and even in world) since most models are based on historical records. Based on the experience gained during MODIRISK a cost-effective sampling strategy will be designed for use in follow-up and similar studies. Modelling will mainly assist in defining the minimal field sample needed to produce acceptable distribution maps, and how these samples are best distributed in space. Furthermore it contributes to the development of state of the art scientific tools integrating collection-based information technology at various resolutions with geographic mapping efforts and remote sensing driven continuous distribution models. This enables to better describe the spatial distribution of mosquito biodiversity, and to understand how it is organized in communities and habitats.

The filling of an essential knowledge gap in Europe, and the expansion of model outputs through linking up with a project in The Netherlands, enables the project to produce more robust results and to prepare better for later expansion of activities in Europe. MODIRISK plays its role as interplay between newly gained insights and the end-users. MODIRISK participated to the risk assessment group of the Scietific Institute of Public Health concerning the presence of the exotic vector species *Oc. j. japonicus* and participated to meetings of the European Centres for Disease Prevention and Control (ECDC). Links were made with the Belgium Forum on Invasive Species by participating to the discussions on the 'Guidelines for environmental impact assessment and list classification of non-native organisms in Belgium" and by acting as member of the scientific committee of the 'Science Meeting Aliens" conference on biological invasion (11th of May 2009). MODIRISK was consulted by the AGORA project on 'set-up of monitoring of potential effects of climate change on human health and on the health of animals'. In January 2009 MODIRISK organised a workshop on vector control in Belgium bringing together persons potentially involved in the decision making process on vector control, other stakeholders and interested persons. A strong plea was made to set-up entomological surveillance to follow the situation and to evaluate the spread of the exotic species in Belgium. Moreover a flow chart of competences and responsibilities of the different in authorities potentially involved in vector control should be established.

The project directly contributes to discovering biodiversity and monitoring/predicting its changes, and actively prepares to address issues such as the assessment of impacts of biodiversity change with particular reference to new invasive mosquito species and the risk to introduce new pathogens. An improved understanding of the biodiversity of mosquito vectors is an essential step towards an improved understanding of the ecology of the diseases they transmit.