Effectiveness of an Integrated Vector Management control strategy for the tiger mosquito *(Aedes albopictus)*: a case study in Sant Cugat del Vallès (Barcelona)

Gisela Chebabi Abramides

A thesis submitted for the degree of Doctor of Environmental Sciences

Supervised by

Dr. Nuria Giménez Gómez   Dr. Sybille nan den Hove   Dr. David Roiz

September 2012
ICTA Universitat Autònoma de Barcelona

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Agraiments

Aquest treball ha sigut possible gràcies al suport i la col·laboració de molta gent.

Agraeixo als meus tres directors Nuria Giménez, David Roiz i Sybille van den Hove per la confiança que han tingut en el meu treball des del començament. Tres persones brillants que durant aquests anys m'han donat suport incondicional en molts aspectes. M'agradaria fer un agraiment especial a la Nuria, que em va obrir moltes portes, gràcies pels dinars i berenars, per a mi han sigut moments màgics que hem compartit (juntament amb el mosquit).

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> "Knowing is not enough; we must apply.
> Willing is not enough; we must do."
>
> *Goethe*

Effectiveness of an Integrated Vector Management control strategy for the tiger mosquito (*Aedes albopictus*): a case study in Sant Cugat del Vallès (Barcelona)

Abstract

The Asian tiger mosquito, *Aedes albopictus* (Skuse, 1894), is an invasive species that is currently found in the five continents, spreading from its native area in Southeast Asia through the international trade of used tires and lucky bamboo. In Catalonia it was first reported in 2004 in the municipality of Sant Cugat del Vallès, and has quickly expanded over the last five years to more than 200 municipalities in Catalonia and Alicante. This species is a vector of several arboviruses, and it has been associated with the transmission of dengue and chikungunya virus in several areas of the world causing serious concerns to people and public health authorities. In Europe, the outbreak of chikungunya fever in Italy during 2007, and subsequent autochthonous cases of dengue fever in France and Croatia in 2010, raised the urgency of establishing effective surveillance and control programs for the vector.

The objectives of this study were: (1) To evaluate the effectiveness of an Integrated Vector Management (IVM) control strategy of the *Aedes albopictus* population in Sant Cugat del Vallès; (2) to determine the several key factors affecting mosquito populations in the premises inspected during the "door-to-door" campaigns; (3) to describe the larval containers preferred by the species; (4) to explore the perception and the involvement of the population of Sant Cugat in the control of this invasive species and (5) to propose a standard protocol towards an IVM control strategy at local level.

During 2008-2010 an IVM campaign was performed in six neighbourhoods of Sant Cugat del Vallès. The IVM strategy included source reduction campaign (with door-to-door visits), larvicide treatments (*Bacillus thuringiensis israelensis* and diflubenzuron), adulticide treatments (alpha-cipermetrin) and cleaning up uncontrolled landfills. The effectiveness of the introduction was evaluated through

Generalised Linear Models based on the differences in the abundance of mosquitoes monitored through oviposition traps. In addition in 2010 a larval survey was carried out.

In total 3720 dwellings were visited and 820 householders were interviewed. The results showed the number of eggs significantly reduced in the intervened areas in 2008-2009 when compared to the control ones. In 2008, the accumulate median of eggs was 175 and 272 in the intervention and control areas, respectively. In 2009, these medians were 884 and 1668 eggs. In the three study years an association was observed between some environmental factors, such as premises with vegetable garden (15%; CI 95%: 9-20%), stacked materials (15%; CI 95%: 9-20%), coops (5%; CI 95%: 2-8%) as well as commercial premises (11%; CI 95%: 7-15%), and sites with works (3%; CI 95%: 1-6%), with the detection of larval breeding containers.

The larval density surveys highlight small containers (≤0.5 l) as preferred breeding sites for this species. In the peridomestic areas of the inspected premises, containers as scuppers, drums, solid waste and buckets were frequently found with *Aedes albopictus* larval breeding.

Citizen cooperation proved to be an essential factor for success. A high level of collaboration by the home onwers, who allowed entry into their private dwellings was attained. These findings suggest that the strategy was effective in reducing the number of eggs. Finally, we propose a protocol building on our practical experience and research on the control of this species over the years of this study. This work might be a model for controlling the populations of *Ae. albopictus* in other municipalities of the Mediterranean region.

Efectividad de una estrategia de Gestión Integrada de Vectores para el control del mosquito tigre (*Aedes albopictus*) en el área de Sant Cugat del Vallès (Barcelona).

Resumen

El mosquito tigre, *Aedes albopictus* (Skuse, 1894), es una especie invasora que actualmente se encuentra en los cinco continentes, extendiéndose desde su área nativa en el sudeste de Asia a través del comercio internacional de neumáticos usados y del bambú de la suerte. En Cataluña fue detectado por primera vez en el año 2004, en el municipio Sant Cugat del Vallès, y se expandió rápidamente en los últimos cinco años a más de 200 municipios de Cataluña y Alicante. Esta especie es un vector de diferentes arbovirosis, y ha sido asociada con la transmisión del virus del dengue y la chikungunya en varias zonas del mundo, causando una gran preocupación a las personas y las autoridades de salud pública. En Europa, el brote de fiebre chikungunya que ocurrió en Italia en 2007, y los posteriores casos autóctonos de dengue en Francia y Croacia en 2010, acentuaron la necesidad de establecer programas efectivos de vigilancia y control del vector.

Los objetivos de este estudio fueron: (1) Evaluar la efectividad de una estrategia de gestión integrada de vectores para el control de la población de *Aedes albopictus* en Sant Cugat del Vallès (2), determinar algunos factores que afectan las poblaciones de mosquitos en los domicilios inspeccionados durante las campañas "puerta-a-puerta", (3) describir los recipientes preferidos por las larvas de la especie, (4) analizar la percepción y la participación de la población de Sant Cugat en el control de esta especie invasora y (5) proponer como estrategia de control un protocolo específico para la localidad.

En total se visitaron 3.720 viviendas y se entrevistó a 720 personas. Además durante 2008-2010 se aplicó en seis barrios del área un programa de gestión integrada para controlar al mosquito tigre. La estrategia incluyó la reducción de los focos de cría (con visitas puerta-a-puerta), los tratamientos larvicidas (*Bacillus thuringiensis israelensis* y diflubenzuron), tratamientos adulticidas (alfa-cipermetrin) y la limpieza de los vertederos incontrolados. En 2010 también se realizó un estudio de las densidades de larvas en los recipientes donde fueron

detectadas. La efectividad de la introducción se evaluó con un modelo lineal generalizado donde la variable dependiente fue la abundacia de huevos de mosquitos monitorizados a través de trampas de oviposición.

Los resultados mostraron un número significativamente inferior de huevos en las áreas intervenidas en 2008-2009 comparadas con las áreas control. En 2008, las medianas acumuladas de huevos fueron respectivamente de 175 y 272 en las zonas de intervención y control; en 2009, estas medianas fueron de 884 y 1668 huevos. En los tres años estudiados se observó relación entre algunos factores ambientales y una mayor detección de recipientes con crecimiento de larvas destacando: las instalaciones con huertas (15%; IC 95%: 9-20%), materiales apilados (15%; IC 95%: 9-20%), locales comerciales (11%; IC del 95%: 7-15%), gallineros (5%; IC 95%: 2-8%) y obras (3%; IC 95%: 1-6%).

En el estudio de la densidad larvaria, destacaron los pequeños contenedores (≤0,5 l) como preferidos para el desarrollo de la especie. Imbornales, bidones, residuos sólidos y cubos fueron frecuentemente detectados con crecimiento de larvas de *Aedes albopictus*.

La cooperación ciudadana, un factor esencial para el éxito, fue elevada gracias a la colaboración de los propietarios de las casas que facilitaron el acceso al interior de sus viviendas privadas. Estos hallazgos sugieren que la estrategia fue efectiva para disminuir el número de huevos. Finalmente, presentamos un protocolo basado en en la estrategia utilizada para controlar esta especie durante estos años. Este trabajo podría utilizarse como modelo para controlar las poblaciones de *Ae. albopictus* en otros municipios de la región del Mediterráneo.

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List of acronyms.

BI – Breteau Index

Bti – *Bacillus thuringiensis israelensis*

CDC – Centers for Disease Control and Prevention

CHIK – Chikungunya

CHIKV – Chikungunya virus

CI – Confidence Interval.

CI – Container Index

DFB – Diflubenzuron

DEN – Dengue

DENV – Dengue virus

DHF – Dengue hemorrhagic fever

DDT – Dichlorodiphenyltrichloroethane

DSS – Dengue Shock Syndrome

ECDC – The European Centre for Disease Prevention and Control

EMCA – European Mosquito Control Association

EVITAR – Infectious Diseases Virosis Transmitted by Arthropods and Rodents Network

GARP – Genetic Algorithm for Rule Set Production

GLM – Generalized Linear Model

GIS – Geographic information systems

GPS – Global Positioning System

HI – House Index

IAEA –International Agency of Atomic Energy

ICTA – Environmental Science and Technology Institut

IGRs – Larval Growth Regulators

IMS – Invasive Mosquito Species

IPCC – Intergovernmental Panel on Climate Change

ISSG – Invasive Species Specialist Group

IUCN – World Conservation Union

IVM – Integrated Vector Management

RIDL – Release of Insects Carrying a Dominant Lethal

SCM – Mosquito Control Services

SIT – Sterile Insect Technique

SR – Source Reduction

SSC – Species Survival Commission

ULV – Ultra Low Volume

VBD – Vector Borne Disease

VBORNET – European Network for Arthropod Vector Surveillance for Human Public Health

WHO – World Health Organistaion

YF – Yellow Fever

Chapter 1
General Introduction

1.1. Concerns about the Asian tiger mosquito

The control of the Asian tiger mosquito *Aedes* (*Stegomyia*) *albopictus* (Skuse 1894) (*Diptera: Culicidae*) is a challenge that has concerned public health authorities worldwide. Globalization and the transport of goods across continents have contributed to the continuous expansion of this invasive insect over the last 30 years. A native species of Southeast Asia, the tiger mosquito has currently colonized countries on 5 continents, causing great discomfort and endangering people's health. Efforts made so far have been shown to be insufficient to stop the spread of this mosquito (Paupy *et al.* 2009, Enserink 2008).

Despite the fact that the Asian tiger mosquito originally used to live at the edges of forests, breeding in tree holes and other natural reservoirs, in the newly infested areas this invasive mosquito has adapted easily to the human peridomestic environment, where pots, vases, drums and buckets can be breeding sites (Rezza 2012, Gratz 2004, Knudsen 1995).

In 2004 the Asian tiger mosquito *Aedes albopictus* was detected in Spain, in the municipality of Sant Cugat del Vallès, province of Barcelona (Aranda *et al.* 2006), becoming the major urban pest in the region and considerably affecting the quality of life of the residents (Curco *et al.* 2008). At the time of writing, this invasive species is known to have expanded its distribution to 210 Catalonian municipalities.

The tiger mosquito has the potential to colonize the Mediterranean basin (Caminade *et al.* 2012, Benedict *et al.* 2007, Eritja *et al.* 2005; Mitchell 1995), and has been reported from most European Mediterranean countries, (Gatt *et al.* 2009, Wymann *et al*. 2008, Scholte & Schaffner 2007, Klobučar *et al*. 2006-a, Aranda *et al*.

2006, Petrić *et al*. 2006, Samanidou-Voyadjoglou *et al*. 2005, Schaffner *et al*. 2004, Flacio *et al*. 2004, Schaffner & Karch 2000).

Both in the field and in the laboratory, this species is an efficient vector of several arbovirosis, such as Chikungunya (CHIK), Dengue (DEN) and Dirofilaria (Medlock *et al.* 2012, Talbalaghi *et al.* 2010, Paupy *et al.* 2009, Hubaleck 2008, Vazeille *et al*. 2008, Cancrini *et al*. 2007, Fontenille *et al.* 2007, Gratz 2004).

Controlling the expansion and abundance of the tiger mosquito is essential because it is the principal way to prevent the transmission of emerging arbovirosis, which are recurrently introduced into Europe by travellers (Burdino *et al.* 2011, Sanchez-seco *et al*. 2009, Beltrame *et al.* 2007, Depoortere *et al*., 2006). It is also important to avoid the nuisance and reduction in quality of life of people in areas where currently there is no disease; however this is a challenging task. At present there have been only a few examples of successful *Aedes* spp. control, (Abramides *et al*. 2011, Paupy *et al.* 2009, Kittayapong *et al.* 2008) and until now the only achievement has been a transitory density control (Richards *et al.* 2008, Kay *et al.* 2005).

Integrated Vector Management (IVM) which includes interventions that use an educational program and chemical or biological methods of vector control is currently considered the most effective strategy to reduce the density of *Aedes spp.* mosquitoes in an area (Esu *et al.* 2010, Ballenger-Browwning & Elder 2009; Kittayapong *et al.* 2008, Erlanger *et al.* 2008).

1.2. Objectives

The central question of this thesis was to evaluate the effectiveness of a multiple interventions strategy to reduce the abundance of the Asian tiger mosquito in an area where it is firmly established. It was decided to use the Integrated Vector Management (IVM), which consists of the application of combined interventions.

The thesis aimed to achieve the following objectives:

1 - To evaluate the effectiveness of an Integrated Vector Management (IVM) control strategy of the *Aedes albopictus* population in Sant Cugat del Vallès. The

IVM strategy consisted of the combination of four actions: source reduction; larvicide treatments (*Bacillus thuringiensis israelensis* and diflubenzuron); adulticide treatments (alpha-cipermetrin); and cleaning up uncontrolled landfills.

2- To determine several key factors affecting mosquito populations in the premises inspected during the “door-to-door” campaigns (for source reduction)

3 - To identify describe the larval containers preferred by the species.

4- To explore the perception and the involvement of the population of Sant Cugat in the control of this invasive species.

5- To propose a standard protocol towards an IVM control strategy at local level.

To address the objectives of this study an extensive literature review was made.

Different actions were proposed and performed in the field during the years 2008-2010. Door-to-door visits, larvicide treatments, clearance of waste and ultimately insecticide spraying were the combined intervention implemented in the study zones.

To measure the efficacy of the control strategy, the relative abundance of mosquitoes was assessed using ovitraps in different control and intervened areas. Before our study was carried out, to the best of our knowledge, there was no evidence in the Mediterranean area on the effectiveness of Integrated Vector Management (IVM) for reducing mosquito abundance in an infested zone.

The interventions were performed through a partnership between the Universitat Autònoma de Barcelona and the Sant Cugat city administration. The monitoring program was carried out by the municipality of Sant Cugat. However, the Regional government of Catalonia (Generalitat de Catalunya) also monitored the territory with the installation of traps, and the rural guards carried out the maintenance of those traps during their daily routes, under the supervision of the technical members of the Environment Department of the Generalitat.

1.3. A case of interdisciplinary issue-driven environmental research

As with many research studies in environmental science, the science developed here (and the science on which it builds) has direct policy relevance. Health and environmental authorities need to address the socio-environmental health problem posed by the invasion of the tiger mosquito in Europe. This requires effective early warning, surveillance and control of vectors, based on extensive scientific understanding of various aspects of the biology and ecology of the species, but also grounded on social sciences understanding of the socio-economic and cultural context and elements influencing public response to any policy process. Hence on the research side, it is essential to address the issue through an interdisciplinary approach.

In this endeavour the candidate has been helped by her own interdisciplinary background, and by that of its supervisors, as individuals or as a group. Similarly, the policy process needs an intersectoral involvement, which is fundamentally to support interdisciplinary and integrated vector management policies and their implementation (Medlock *et al*. 2012, Braks *et al.* 2011; WHO 2008). In this manner, this work continuously operated at the interface between intersectoral policy and interdisciplinary science, with the ultimate objective to contribute to combating the inconvenience and dangers to people's health caused by the invasion of the Asian tiger mosquito.

1.4. Structure of the thesis

The work is structured in 6 chapters:

Following this Introductory chapter, Chapters 2 and 3 are synthesis based essentially on bibliographic desk studies. Chapter 2 describes the key issues related to the global situation of the tiger mosquito, reviewing its vectorial characteristics and the diseases it can transmit, the possible alterations that climate change could cause on the spread of the species and emergence of these diseases. Other invasive mosquitoes and their distribution in Europe are examined, with a special attention given to *Aedes aegypti*, recently reported in Madeira Island (Portugal), the main vector of Dengue fever worldwide.

Chapter 3 contains an in depth revision of surveillance and control methods currently available. Integrated Vector Management, which is considered the more sustainable method for long-term control, is described in this chapter.

The case study of Sant Cugat del Vallès, which represent the core empirical part of the thesis in presented in chapter 4. It describes the reasoning for choosing the methodology as well as all the interventions carried out during the three study years; as well as the relevant findings. Discussion of the value of these findings and possible applications in other regions ensues.

Chapter 5 is a direct application of the findings of chapter 4. Building on the empirical and theoretical experience gained in the project, a protocol for the control of the tiger mosquito in Sant Cugat del Vallès has been designed and is presented in this chapter. A calendar including all the actions and the main tools in both educational outreach and surveillance/control areas is detailed. The possibilities of adapting the protocol to other municipalities are analysed and the advantages and limitations are discussed.

Chapter 6 is a final reflexion of what has been achieved in relation to the objectives of the study. The contributions of the findings to science and to policy support and implementation by health authorities are evaluated, also in terms of shortcomings and limitations, and potential practical implications and interesting avenues for future research have been explored.

Currently there is one publication derived from this thesis, it is attached to this monograph in Annex 1. Two more manuscripts are being finalised to be submitted for publication. One refers to the analysis of the outcomes of the house-to-house visits and surveys: during the 3 studied years more than 3700 visits were carried out with 820 answered questionnaires. The other is an article presenting the proposed protocol for a municipal action to address the challenges caused by the tiger mosquito. Annex 2 contains the inspections sheet, questionnaires and the printed material used in the campaigns.

In Annex 3 the protocol, currently implemented in Sant Cugat del Vallès, is attached (in the original language, Catalan).

Chapter 2.

The history of a stowaway species: *Aedes albopictus*

2.1. The Asian tiger mosquito background

The Asian tiger mosquito *Aedes* (*Stegomyia*) *albopictus* (Skuse 1894) (Diptera: Culicidae), originally from Southeast Asia*,* is an invasive species that in recent decades has spread its range across many temperate and tropical countries. Ranking among the first 100 "World's Worst" invasive especies, according to the Global Invasive Species Database(http://www.issg.org/database/species/search.asp?st=100ss&fr=1&str=&lang=EN) (last consulted August 2012), the insect has been transported passively through international trade, principally in used tires and aquatic plants, with an onward spread within Europe through ground transport (Medlock *et al.* 2012, Enserink 2008, Benedict *et al.* 2007). The insect has colonized all continents including the Mediterranean region (Gratz 2004, Delauney *et al.* 2009, Paupy *et al*. 2009), which seems to have high suitability, especially in the coastal areas (Caminade *et al.* 2012)

Aedes albopictus is a synanthropic and daytime biting species, its use of artificial containers as breeding sites was one of the reasons that contributed to its rapid geographic spread (Paupy *et al.* 2009). It was detected in Spain for the first time in Sant Cugat del Vallès, Catalonia, during the summer of 2004 (Gimenez *et al.* 2007, Aranda *et al*. 2006).

Dangerous because of its potential health implications (Paupy *et al.* 2009, Weaver and Reisen 2010), *Ae. albopictus* is an efficient vector in the field of several arbovirosis (Gratz 2004), Chikungunya (CHIK) and dengue (DEN) are currently the most important diseases that could undergo outbreaks in Europe (Fontenille *et al*. 2007; Vazeille *et al*. 2008; Talbalaghi *et al*. 2010). Also, it is a vector of several other types of encephalitis (Medlock *et al.* 2012, Weaver and Reisen 2010, Gratz 2004, Mitchell 1995) and filariasis (Metlock *et al.* 2012, Cancrini *et al*. 2007, Gratz 2004).

Mosquitoes cause more human suffering than any other organism - over one million people worldwide die from mosquito-borne diseases every year (Reiter 2010, Ferrar *et al.* 2007). DEN is considered the most important arboviral disease in the world, affecting 50-100 million people every year, while over half of the world population is at risk of contracting the disease (Wilder-Smith *et al.* 2012, Reiter 2010, Ferrar *et al.* 2007).

An outbreak of CHIK fever in the summer of 2007 in northeast Italy, with 200 diagnosed cases (Rezza 2007), was the first confirmation that temperate *Ae. albopictus* populations could transmit a tropical virus imported by travellers and cause an epidemic in a European area colonized by the mosquito, creating an important health concern. Since then, two more autochthonous cases have been diagnosed in France in 2010 (Gould *et al.* 2010). Also isolated native cases of DEN were diagnosed in France in 2010 (Gould *et al.* 2010), and in Germany and Croatia (Gjenero-Margan *et al.* 2011, Schmidt-Chanasit *et al.* 2010).

The outbreak of autochthonous chikungunya fever in humans in temperate zones has raised serious concern among health authorities as the potential vector of the viruses, *Aedes albopictus*, is known to be spreading widely throughout many regions of southern Europe (Enserink 2008, Vazeille *et al.* 2008). Currently no transmission of arboviruses has been reported in Catalonia, but the potential risk exists: during 2006-2007 it was detected in Spain that 9.4% of travellers returning from endemic areas with fever or joint pains, were CHIK positive (Sanchez-Seco *et al.* 2009).

Integrated vector management (IVM) techniques, that includes source reduction (SR, a community-based approach), pesticide application, biological control, education programs and public awareness, as well as personal protection are currently considered the most effective strategy to reduce the density of mosquitoes in an area (Medlock *et al.* 2012, Ballenger- Browning & Elder 2009; Kittayapong *et al*. 2008; Erlanger *et al*. 2008; Esu *et al*. 2010). Community-based interventions tend to be sustainable as they aim to change behaviour and induce social mobilization (Pérez *et al.* 2007).

The European Centre for Disease Prevention and Control (ECDC) has a responsability to identify, assess and communicate current and emerging dangers to human health by infectious disease through the use of monitoring and surveillance tools (Braks *et al.* 2011). Thus, ECDC created VBORNET, a network of medical entomologists and public health experts which addresses the essential issues for vector surveillance activities of arthropods in the European Union. ECDC also organizes meetings of an expert group on climate change to analyse its effects on human health, the last report was published in February of 2012 and can be accessed by the link:
http://ecdc.europa.eu/en/publications/Publications/120223-Climate-change-meeting-report.pdf (Last consulted August 2011).

Besides, the European Mosquito Control Association (EMCA), which promotes the improvement of the quality of life of the European population by reducing mosquito nuisance level, has been organizing workshops and gathering of professionals and experts from Europe and other continents.

Recently, a complete guidelines document (Guidelines for the surveillance of invasive mosquitoes in Europe) was created for surveillance of invasive mosquito species in the continent in order to further harmonize monitoring procedures and to give practical tools and accurate information to support Member states of European Union their decision making. The guidelines are available on line: (http://ecdc.europa.eu/en/publications/Publications/TER-Mosquito-surveillance-guidelines.pdf) (last consulted September 2012)

Early detection of invasive mosquitoes increases the opportunity for appropriate and timely response measures and therefore mosquito-borne disease (ECDC 2012 - Guidelines for the surveillance of invasive mosquitoes in Europe).

Despite all the efforts to stop the expansion of this invasive species, when the tiger mosquito is established in a zone, it has up to now been impossible to eradicate and very difficult to reduce the size of its population (Enserink 2008).

2.2. Origin and current dispersion

Originally *Aedes albopictus* was found in some islands of the Indian Ocean, in various countries in the oriental region of Asia, and in Madagascar. It was present in China northward to Beijing (Feng 1938), in Korea to about the latitude of Seoul and in Japan (Huang 1968, Tanaka *et al.* 1979). In the last century, the distribution of *Ae. albopictus* has expanded eastward to Hawaii and islands in the south Pacific (Hawley 1988, Ramalingam 1983, Pashley and Pashley 1983, Elliott 1980, Joyce 1961, Bonnet 1948). *Aedes albopictus*, received the nick-name "Tiger mosquito", in south-east Asia, due to its vivid median silvery line extending from the head to the dorsum and distinct tarsal stripes (Lounibos 2002). In the mid-1980s, however, the picture changed causing an alarming shift in the distribution of the species.

Several small introductions into continental North America were observed (Reiter 1998, Hawley 1988), and in 1985 an important larval habitat of the insect was discovered in Houston, Texas (Sprenger and Wuithiranyagool 1986). *Aedes albopictus* most likely reached North America in shipments of used tires from Japan (Reiter 1988, Hawley 1987), expanding its range extensively along the east coast throughout the southeast, and into the lower Midwest (Moore 1999).

An important infestation was reported in 1986 in and around Sao Paulo, Brazil (Foratinni 1986), apparently originating from tropical Asia (Hawley *et al*. 1987). In Mexico it was detected in 1988 and thereafter the species was detected in several countries of Central America (Honduras, Costa Rica, El Salvador, Panama), and some Caribbean Islands after 1993 (firstly the Dominican Republic, then the Cayman Islands and Cuba). Later, *Ae. albopictus* was also reported from Guatemala and Bolivia (1995), Colombia (1997), Argentina (1998) and Nicaragua (2003) (Eritja *et al*. 2005). Nowadays it is present in all the countries of Central and South America except Chile. In the Pacific area, the invasive species was reported in Salomon, Australia (1988), New Zealand (1994), and La Réunion (1994).

In African countries it was found in South Africa (1990), and now it is well established in Nigeria, Gabon, Equatorial Guinea and Cameroon (Paupy *et al.* 2009, Simard *et al.* 2005, Fontenille and Toto 2001), Equatorial Guinea (Toto *et al*. 2003, Krueger and Hagen, 2007). In the Middle East *A. albopictus* is present in Israel (Pener *et al*. 2003), Lebanon and Syria (Haddad *et al*. 2007). This species is also spreading across the Pacific islands of Palau, Yap and Fiji (CDC 2005) and Torres Strait Islands in Australia (Ritchie *et al*. 2006).

In Europe, *Ae. albopictus* was first found in Albania in 1979 (Adhami and Murati 1987), but concern rose when the species was detected in Genoa, Italy in 1990 (Sabatini *et al.* 1990) and one year later near Padua (Dalla Pozza and Majori 1992). Since then, the species spread its range across several regions of Italy, and is currently established in almost all of them (Romi *et al.* 2008). Genetic analysis associates affinities between Italian, US and Japanese specimens of *Ae. albopictus* (Urbanelli *et al.* 2000).

In France it was detected in 1999 in two tire dumps (Schaffner and Karch 2000; Schaffner *et al*. 2001. The next detection was in Belgium (Schaffner *et al.* 2004) tracing back the route of infested shipments from France. Later, the species has been reported in from Montenegro (Petrić *et al*. 2001) and from discarded tires in Israel (Pener *et al*. 2003). Detection reports of *Ae. albopictus* have not always have been followed by establishment, recent infestations in the Netherlands have been traced to imports of 'lucky bamboo' (*Dracaena sanderiana*) from sub-tropical China, but these mosquitoes do not appear to have survived the winter, perhaps because of the absence of winter diapauses (Reiter 2010, Scholte *et al*. 2007).

The current spread and current expansion of the tiger mosquito in Europe are summarised in Table 1 and in Figures 1 a-b

Table 1 – Expansion of the tiger mosquito in Europe and situation of surveillance and control.

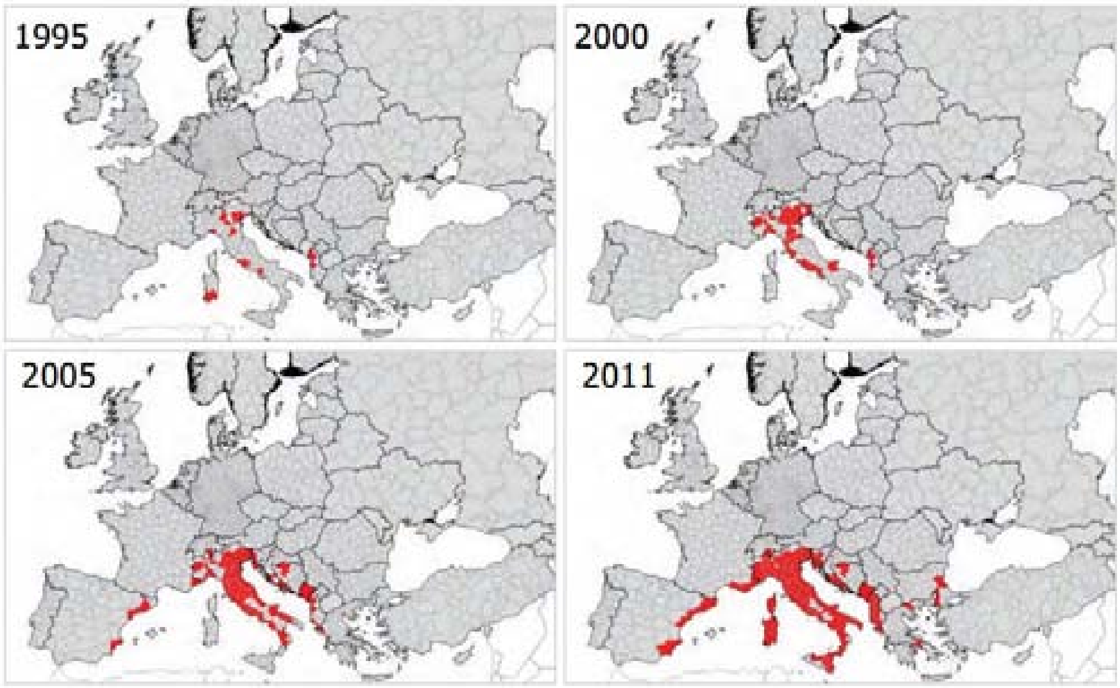
Country	Detection	Surveillance	Control
Albania	Adhami & Murati (1987); Adhami & Reiter (1998)	No surveillance or studies	-
Italy	Sabatini *et al.* (1990)	Surveillance local administrations	Control of *Aedes albopictus* in Italy occurs almost exclusively at locations where the species is abundant and causes biting nuisance
France	Schaffner and Karch (2000)	Regional Surveillance local administrations	Local authorities funds routine mosquito control
Belgium	Schaffner *et al*. (2004)	Surveillance country wide	-
Montenegro	Petrić *et al*. (2001)	No surveillance or studies	-
Serbia and Montenegro	Petrić *et al*. (2001) Schaffner *et al.* 2003	No surveillance or studies	
Switzerland	Flacio *et al.* (2004); Wymann et al. (2008)	At local and national levels	In Ticino, since 2004
Greece	Samanidou-Voyadjoglou *et al*. (2005), Patsoula et al 2006	Regional surveillance	Regional control
Spain	Aranda *et al*. (2006)	Regional surveillance (Catalonia)	Regional monitoring (traps), county and local administration control
Croatia	Klobučar *et al.* (2006b)	Regional levels	Infested coastal areas
The Netherlands	Scholte *et al.* (2007)	National level since 2006	Applied by greenhouse owners
Bosnia and Herzegovina	Personal communication, Z. Lukac, in Petrić et al. (2006)		Only in one canton (Banja Luka)
Slovenia	Petrić *et al.* (2006)	Regional Surveillance in 2007 and 2010 (Kalan et al 2011)	Monitoring of southwestern part of the country.

Table 1 - continued

Monaco	ECDC 2009	National programme against Culex not against Aedes albopictus	-
Germany	Pluskota *et al.* (2008)	Surveillance and control programmes for mosquitoes are implemented in a few areas	Started in 2008
San Marino	(ECDC 2009)	-	-
Vatican City	(ECDC 2009)	-	-
Corsica	(Scholte and Schaffner 2007)	-	-
Malta	Gatt *et al*. 2009	-	-
Bulgaria	Caminade *et al.* 2012	-	-
Israel	Pener *et al*. 2003	-	-
Turkey	Caminade *et al.* 2012	-	-

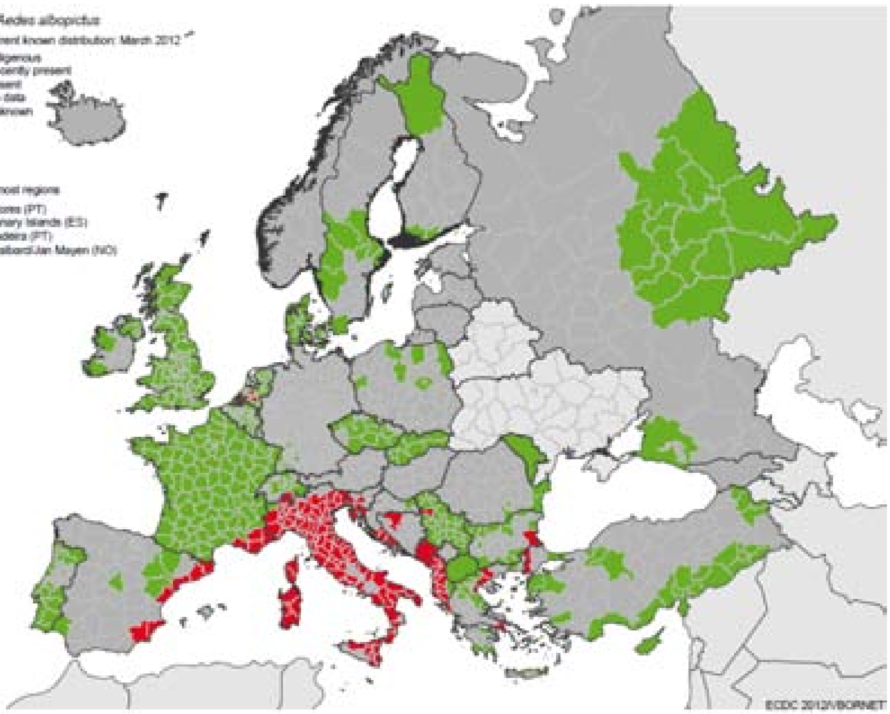
Table 2 - Update from ECDC - Development of *Aedes albopictus* risk maps 2009 (http://www.ecdc.europa.eu/en/publications/publications/0905_ter_development_of_aedes_albopictus_risk_maps.pdf)

Figure 1-a – Spread of the Asian tiger mosquito *Ae. albopictus* in Europe, 1995–2011.



Source: Guidelines for the surveillance of invasive mosquitoes in Europe (ECDC 2012)

Figure 1-b – Known distribution of *Ae. albopictus* based on field observations.



Source: ECDC Vebornet - based on field observations from the ECDC/VBORNET project (December 2011).

During recent years several studies have been carried out using different modelling approaches and tools, such as Genetic Algorithm for Rule Set Production (GARP) and Geographic Information Systems (GIS) to generate risk maps in order to analyse the impact of climatic and photoperiodic thresholds on areas of establishment of the tiger mosquito, predicting the continued spread of the species (Caminade *et al.* 2012, Neteler *et al*. 2011, Roiz *et al*. 2011, Fisher *et al.* 2011, ECDC 2009, Benedict *et al.* 2007, Scholte and Shaffner 2007, Medlock *et al.* 2006).

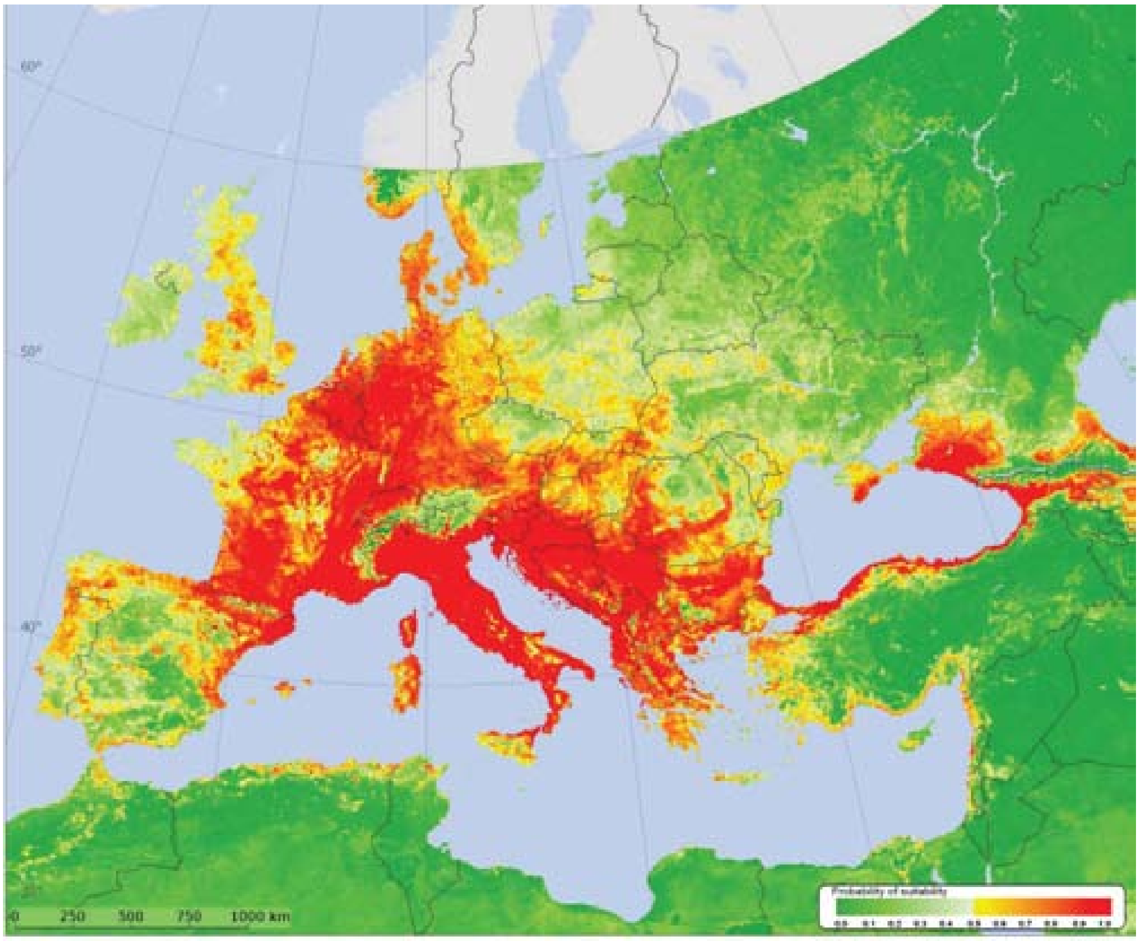
The ECDC has been working for the development of tiger mosquito distribution risk maps with different models according to the climatic suitability for the establishment of the insect and dengue transmission in Europe. The use of datasets for climate, environmental conditions, geography, population density, and mathematical modelling (nonlinear discriminant analysis) was conducted to assess the areas of Europe most climatically suitable for *Ae. albopictus*, *Ae. aegypti*, and dengue transmission.

Models were run for each of the individual *Ae. albopictus* databases and maps were produced based upon averages from 100 bootstrap models. The outputs from all of these models were then combined to produce the final map (Figure 2), which presents the estimated areas of environmental suitability for *Ae. albopictus* in continental Europe (ECDC – Climate Change Technical Report - 2012).

Caminade *et al*. (2012) also considered the recent climate change context and the regional climate models to evaluate how suitability for the mosquito might change in the near future (e.g. 2030–2050). According to these analysis, the multi model approach shows that the future climatic hot spots for *Ae. albopictus* are Portugal, the southern UK, the Benelux, western Germany, Slovakia, Cyprus, Macedonia, Bulgaria, Slovakia, Hungary and Turkey (Caminade *et al.* 2012).

The availability of predictive models for the risk of establishment of the vector could contribute substantially to prediction of infectious disease in uninfected regions, including Europe.

Figure 2 – Climatic suitability for *Ae. albopictus* in Europe.



This map was produced by combining the outputs of all the Ae. abopictus models using a non-linear discriminant analysis. The probability scale is from zero to 1.0: Probabilities from [0–0.49] are coloured green (darker to lighter) and indicate conditions not suitable for the vector (or gradual predicted absence of the vector). Probabilities from [0.5–1] are coloured yellow through to red, indicating conditions increasingly suitable for the vector (or gradual predicted presence of the vector)

Source: ECDC 2012 - The climatic suitability for dengue transmission in continental Europe. 2012 (http://ecdc.europa.eu/en/publications/Publications/TER-Climatic-suitablility-dengue.pdf) (last consulted August 2012)

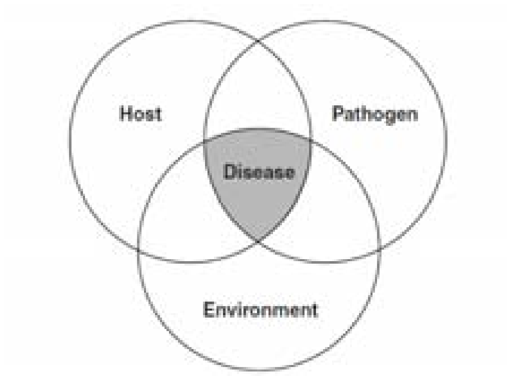
2.3. Vector-Borne Diseases (VBDs)

Vector-borne disease (VBD) happens when a pathogen agent is transmitted between vertebrate hosts by another organism, the vector. VBD is called arbovirosis in the case of arthopod vectors, like mosquitoes and other biting flies, and ticks (Braks *et al.* 2011, Weaver and Reisen 2010).

Mosquitoes (Diptera, culicidae) are the best known group of insects which are vectors of diseases, standing out as clearly opportunistic organisms able to exploit intermittent new environments. There are eco-epidemiological factors in the natural history of mosquito-borne viruses that can affect their circulation in natural foci, affecting primarily their vectors (mosquitoes). For instance, major factors are changes in land use and climatic variables. Abundance of host (vertebrates) and vectors, intense summer precipitations, higher temperatures and drought, floods, appropriate habitats, humid building basements and also the contact transmission are some favourable ecological factors for imported or indigenous mosquito borne viruses (Braks *et al.* 2011, Lamballerie *et al.* 2008).

The presence of the pathogen is a necessary but not sufficient cause of a particular disease. The concept of the "epidemiological triad" (host-pathogen-environment) is illustrated in the diagram of Snieszco (1974) (figure 3).

Figure 3 – The "epidemiological triad".



Source: Snieszko (1974)

Global change or globalization is a major risk factor because it leads to the expansion of viruses and mosquitoes (Paupy *et al.* 2009). In the last decades, the ease of movement of pathogens between cities, states, countries and continents is directly associated with a dramatic emergence/resurgence of tropical infectious diseases, and reinforces the urgent need to develop and implement new and sustainable prevention strategies (ECDC - Guidelines for the surveillance of invasive mosquitoes in Europe - 2012, Fontenille *et al.* 2007).

There is a combination of on the one hand increased movement of viruses in people among countries and regions, affecting the level of herd immunity to specific virus serotypes in the human population, and on the other hand genetic changes in circulating or introduced viruses that give them greater epidemic potential, thus rendering them more likely to be transmitted from host to host.

Tropical medicine has been improving disease control, primarily by understanding the ecology of certain diseases through field and laboratory research and then using that knowledge to develop and implement prevention and control programs aimed at breaking the transmission cycles at their weakest points (Gubler 2001).

During the 20th century important diseases have been effectively controlled by antibiotics and other new drugs. Malaria had been erradicated from North America and Europe and controlled in other countries of Asia, Pacific area and the Americas. Also progress has been notable in the control of urban Yellow Fever (YF) in Africa and. Medical entomology, which consists of the application and study of arthropod biology of disease transmissions, is an important tool in the public health institutions and governmental policy-makers (Braks *et al.* 2011).

Changes in ecological systems, or perturbations due to changes in virus pathogenicity, host or vector populations can transform imported VBDs into epidemics. A genetically small viral change or the introduction of new strains can increase virulence and viremia levels in vertebrates, or even enhance vector competence (Weaver and Reisen 2010, Paupy *et al.* 2009).

Actions to control some VBDs might preferably be started even when disease is not present yet, indicators may relate also to the presence and abundance of pathogens and vectors. However, the absence of an epidemic disease burden frequently means that it remains just being a threat, and no data or priority settings for the situation are available (Braks *et al*. 2011).

2.3.1. Vector-borne Disease (VBD) transmitted by *Aedes albopictus*

Historically, *Ae. albopictus* was not considered to have a great capacity to transmit pathogens to humans, whereas the species *Aedes aegypti* is regarded as being highly anthropophilic and involved in human-to-human virus transmissions

(Paupy et al 2009). Nevertheless, in the beginning of this century, the tiger mosquito was implicated in in CHIK outbreaks on islands in the Indian Ocean and in central Africa and Italy (Lambrechts *et al*. 2010) increasing considerably the public health concern. Recently a series of reviews of the potential of *Ae. albopictus* as a vector of a large number of arbovirosis have been published (e.g. Medlock *et al.* 2012, Paupy *et al.* 2009, Gratz 2004).

The insect can transmit 26 arboviruses that belong to the *Flaviviridae* (genus *Flavivirus*), *Togaviridae* (genus *Alphavirus*), *Bunyaviridae* (genus *Bunyavirus* and *Phlebovirus*), *Reoviridae* (genus *Orbivirus*) and *Nodaviridae* (genus *Picornavirus*) families in laboratory. In addition, although only a smaller number of viruses were detected (n = 14), viral isolation or detection in the field conditions have been reported, including: six flaviviruses: DEN virus (DENV) (DENV-1, DENV-2, DENV-3, DENV-4, West Nile virus, Japanese encephalitis virus), two alphaviruses (CHIK virus and Eastern equine encephalitis virus) and six bunyaviruses (Potosi virus, Tensaw virus, Keystone virus, La Crosse virus and Jamestown Canyon virus (Paupy *et al*. 2009, Gratz 2004). A resume is shown in Table 2. However, it is important to note that the transmission in laboratory and the detection of these viruses does not provide any conclusive evidence that *Ae. albopictus* plays a significant role in the outbreaks. Currently, the role of *A. albopictus* in the transmission of only DEN virus and CHIK virus has been recognized.

Filarial nematodes such as *Dirofilaria immitis*, *Dirofilaria repens* and *Setaria labiatopapillosa* can be also transmitted experimentally and in the field by *Aedes albopictus* (Paupy *et al*. 2009, Cancrini *et al.* 2007). The insect had been associated with the transmission of filariasis in urban environments of Asia and North America (Konishi 1989, Nayar and Knight, 1999). In Italy this invasive species had been recognized as the vector of *Dirofilaria immitis* and *Dirofilaria repens* in the field (Cancrini *et al.* 2007, Cancrini *et al.* 2003a, 2003b), confirming previous laboratory studies (Cancrini *et al* 1995).

Unlike past epidemics mediated by *Ae. aegypti*, *Ae. albopictus* had a relevant role of the primary CHIK vector during the majority of recent outbreaks, including those on several islands of the western Indian Ocean, parts of India, Singapore, Malaysia, Thailand, Sri Lanka, Gabon, and Italy (Hapuarachchi, *et al.* 2010, , Pagès *et al.* 2009, Delatte *et al.* 2008, Ng *et al.* 2009, Rezza *et al.* 2007, Kumar *et al.* 2007).

Table 2 - Viral and nematodes isolation or detection from *Ae. albopictus* in natural conditions and vector competence for arbovirosis.

Pathogen			Field Isolation	Area	Reference	Infection	Transmission
Virus	**Flaviridae Flavivirus**	- Dengue – 1,2,3,4,	+	Asia, Americas Africa	Paupy *et al.* 2010	+	+
		- Japanise encephalitis	+	Taiwan	Weng *et al.* 1996	+	+
		- St Louis encephalitis				+	+
		- West Nile virus	+	USA		+	+
		- Yellow Fever				+	+
		- Encephalitis virus				+	+
	Togaviridae Alphavirus	- Chikungunya	+	-Africa -Indian Ocean - Asia, Italy	- Paupy *et al.* 2010 - Delatte *et al.* 2008 - Rezza *et al.* 2007	+	+
		- Eastern Equine encephalitis	+	USA	Mitchell *et al.* 1992, Turell *et al.* 1994	+	+
		- Venezuelan Equine encephalitis			Beaman and Turell 1991; Turell and Beaman 1992	+	+
		- Western equine encephalitis				+	+
		- Ross River Virus				+	+
		- Sindis				+	+
		- Mayaro				+	+
		- Getah				+	+
	Bunyaviridae Bunyavirus	- Potosi	+	USA	Mitchell *et al.* 1998	+	+
		- Cache Valley	+	USA	Mitchell *et al.* 1998	?	?
		- Tensaw	+	USA	Gratz 2004	?	?
		- San Angelo				+	+
		- Kaystone	+	USA	Gratz 2004	+	_
		- La Crosse (LACV)	+	USA	Grimstad *et al.* 1889; Gerhardt *et al.* 2001 ; Mitchell *et al.* 1998	+	+
		- Jamestown Canyon	+	USA	Gottfried *et al.* 2002	+	+
		- Trivittatus virus				+	_
		- Oropuche				+	_
	Phlebovirus	- Rift Valley fever				+	+
	Reoviridae Orbivirus	- Orungo virus (Africa)				+	+
	Nodaviridae Orbivirus	Nodamura virus				+	+
Nematodes	**Dirofilaria**	*- D. immitis i D. repens*	+	Asia, Italy USA	Konishi 1989; Nayar and Knight 1999; Cancrini *et al* 2007	+	+

Source: Updated from Paupy *et al*. 2009 and Gratz 2004

Ae. albopictus caused DEN epidemics in 1977 in the Seychelles Islands and La Réunion Islands (Lambrechts *et al.* 2010, Metselaar *et al*. 1980), in 1978 in China, (Qiu *et al.* 1993), in 1981 in Maldive Islands (Lambrechts *et al.* 2010), 2001 in Macao and Hawaii (Almeida *et al.* 2005, Effler *et al.* 2005). Also tiger mosquito was responsible for dengue epidemics in Japan and Taipei during World War II (Hotta 1998).

Although some severe and fatal Dengue haemorrhagic fever/ Dengue shock syndrome (DHF/DSS) cases may have occurred, epidemics caused by vector *Ae. albopictus* were essentially classical dengue fever and epidemics of Dengue Haemorrhagic Fever (DHF) have occurred only in areas where *Ae. aegypti* is detected (Lambrechts *et al.* 2010).

Recently, an outbreak of DEN happened on Reunion Island (from January to April of 2012) with 20 autochthonous cases. *Ae. albopictus* was associated to it, since its density was high enough to allow transmission and the weather of the area is suitable during the whole year (Larrieu *et al.* 2012).

2.3.2. Dengue (DEN) and Dengue haemorrhagic fever/Dengue shock syndrome (DHF/DSS)

Dengue virosis (DEN - family *Flaviviridae* genus *Flavivirus*) is the most rapidly advancing vector-borne disease and unique among the arbovirosis in that it does not require a primitive enzootic forest cycle for maintenance, being fully adapted to the human host and human environment (Reiter 2010, Farrar *et al.* 2007, Gubler 2002).

By the beginning of the twentieth century, dengue was a major public health problem in most tropical countries. However, with the exception of Southeast Asia, epidemic dengue was effectively controlled in most of these countries in the 1950s and 1960s as a side benefit of malaria and yellow fever control programs (Gubler *et al.* 2002).

The reasons for the resurgence and re-emergence of DEN and DHF in the past 30 years are associated with demographic, environmental, and public health changes; globalization, rapid urbanization on a massive scale and global travel have been key factors in this resurgence. (Farrar *et al.* 2007). Dengue is an emerging infectious disease that is estimated to affect 50-100 million individuals each year in tropical and subtropical areas (Rezza 2012), almost 50% of the world's population are currently living with a risk of contracting the disease, and it has become a serious concern among health authorities (Farrar *et al.* 2007).

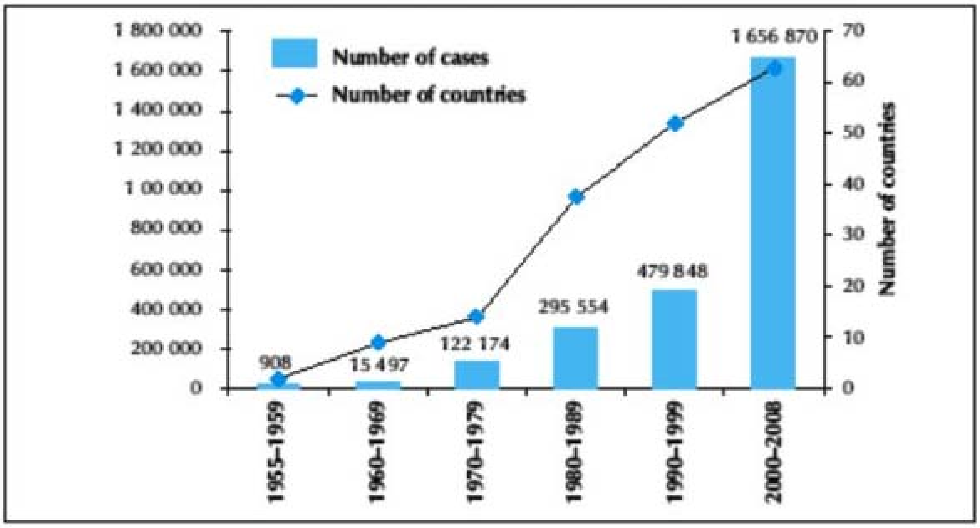
There are four dengue viruses (DEN-1, DEN-2, DEN-3, and DEN-4) that have the same epidemiology and cause similar illness in humans, but are antigenically distinct. All four viruses are now maintained in an endemic cycle involving humans and *Aedes aegypti* mosquitoes in most urban centres of the tropics.

As many as 80% of all dengue infections are asymptomatic and less than 5% can be severe, a fraction of this may be fatal (Farrar *et al.*, 2008). Infection with one serotype provides lifelong homologous immunity only for that serotype, and after a few months, the presence of non-neutralizing antibodies increases the risk for progression to DHF when the patient is infected by any of the other 3 serotypes (Rezza 2012, Gubler *et al.* 2002). Co-circulation of various virus serotypes in a community (hyperendemicity) is the single most common risk factor associated with the emergence of the severe form of disease DHF, the factors responsible for emergence of hyperendemicity associated with periodic epidemics in an area are not well understood (Gubler *et al.* 2002).

Dengue viruses continue to expand their range, as shown by movement of dengue virus type 1 (DENV-1) into Hawaii in 2001–02. This outbreak of dengue was the first in Hawaii since World War II ended (Hayes *et al.* 2006), the virus was transmitted by *Aedes albopictus* (Halstead 2007).

From the beginning of this century, *Aedes albopictus* was the vector of dengue transmission in several outbreaks, like the ones in Hawaii, Mauritius and China (Effler *et al.* 2005, Issak *et al.* 2010, Xu *et al.* 2007). Simultaneous transmission of DEN and CHIK were reported in Gabon and Madagascar (Leroy *et al.* 2009, Ratsitorahina *et al.* 2008). In all those places DEN was absent for a long time. The average number of cases reported to WHO during 1955-2008 are depicted in Figure 4.

Figure 4 - Average annual number of cases of DF/DHF reported to WHO, 1955-2008.



Souce: www.who.int (Last consulted September 2012)

The current world areas of risk of dengue transmission are showed on Figure 5.

Figure 5 – Dengue distribution map.

Source: WHO (2012) International Travel and Health - Disease distribution maps
http://gamapserver.who.int/mapLibrary/Files/Maps/Global_DengueTransmission_ITHRiskMap.png
(Last consulted August 2012)

Lately, partners from several countries of Europe, Asia and South America have created a consortium named Dengue Tools, which aims at achieving better diagnosis, surveillance, prevention, and predictive models and improving the knowledge of the spread of dengue to previously uninfected regions in the context of globalization and climate change. Dengue Tools is an answer to a European Commission's call through the 7th Framework Programme for Research, namely "Comprehensive control of Dengue fever under changing climatic conditions", and comprises several work packages covering research in different areas (Wilder-Smith *et al.* 2012).

2.3.3. Chikungunya Virus (CHIKV)

Chikungunya virus is endemic to Africa, Southeast Asia and the Indian subcontinent. *Aedes aegypti* and *Aedes albopictus* mosquitoes are the main vectors responsible for urban transmission of CHIKV (WHO 2012). Human disease involves the classic triad of clinical symptoms: fever, arthralgia, and maculopapular rash on the face, limbs, and trunk. Arthralgia persists in some patients for weeks or years (Powers and Logue 2007, Pialoux *et al*. 2007).

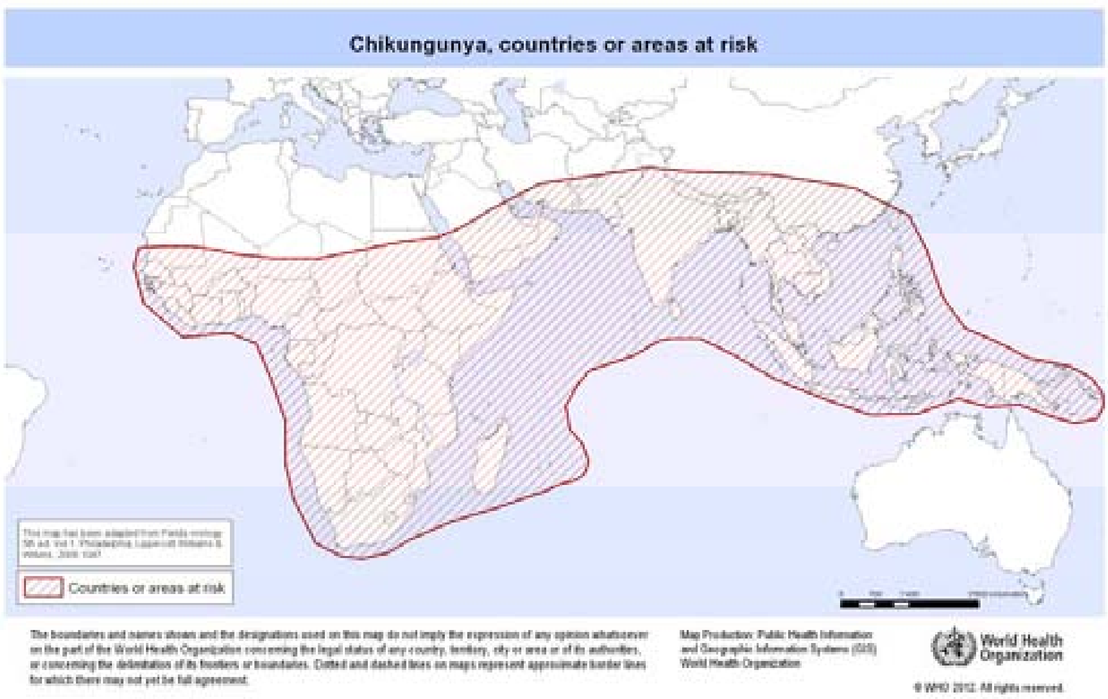
In 2004, CHIKV re-emerged in Kenya and subsequently spread eastward, causing millions of disease cases throughout countries in and around the Indian Ocean and altering the public health infrastructure in these regions (Staples *et al.* 2009). Large CHIKV outbreaks were reported in 2005–2006 in the islands of the Indian Ocean (Renault *et al*. 2007) and the Indian subcontinent (Yergolkar *et al*. 2006).

The outbreak that occurred on Réunion Island (2003) resulted in one third of the population being infected and in 2006–2007, dozens of Chikungunya fever cases were reported among tourists returning from the islands of the Indian Ocean (Réunion, etc.) and the Indian subcontinent to many European countries e.g. France, UK, Germany, Italy, Belgium, Switzerland, Czech Republic, Norway, and Spain (Fontenille *et al*. 2007; Powers and Logue 2007; Sanchez-Seco *et al*. 2009).

In 2007, CHIKV was imported into Europe by an infected person returning from India, causing an outbreak of Chikungunya (CHIK) fever in Italy (Rezza, 2007). Isolated transmissions happened in 2010 in France (Gould *et al*. 2010). This outbreak suggested for the first time the significant potential of the virus to move to novel ecological niches, including in Europe, Australia, and other parts of the Western Hemisphere. This propensity for dispersal and emergence in remote ecological environments illustrates the adaptability of the virus, in particular to new vector populations (Staples *et al.* 2009). Until recently, during human outbreaks, the principal identified vector of CHIKV was *Ae. aegypti*. However, CHIKV has recently been associated with *Ae. Albopictus* due to a virus mutation (Konstantin *et al.* 2012, Lambrechs *et al.* 2010, Weaver and Reiser 2010 ; Delatte *et al.* 2009, Vazeille *et al.* 2008).

The current global areas of risk of CHIKV transmission are depicted in Figure 6.

Figure 6 – Risk areas for Chikungunya transmission.



Source: WHO (2012) International Travel and Health - Disease distribution maps http://gamapserver.who.int/mapLibrary/Files/Maps/Global_Chikungunya_ITHRiskMap.png (Last consulted August 2012)

Currently several regions of Southern Europe have suitable temperatures for CHIK viral transmission and a large number of potentially viraemic visitors come every year (Poletti *et al.* 2011, Vazeille *et al.* 2008, Fontenille *et al.* 2007).

2.3.4. Effects of climate change on mosquito borne diseases

Climate change may happen due to natural internal processes or external forcings, or to persistent anthropogenic changes in the composition of the atmosphere (IPCC 2007). The rapid changes recently observed in the global climate due to anthropogenic emissions of greenhouse gases have caused international scientific concern. With further emissions extreme climate events (heat waves and flooding) are projected to be likely to increase in Europe. In northern parts, winters could become considerably milder with increased precipitation, whereas the southern parts could experience markedly warmer and drier summers (ECDC Climate change meeting report - 2012). Available on line:

http://ecdc.europa.eu/en/publications/Publications/120223-Climate-change-meeting-report.pdf) (Last consulted August 2012).

The Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC) published in 2007 a report (IPCC 2007) addressed the potential effects on health caused by climate change in different parts of the world. In the light of climate change, different hypothesis have been prepared to explain its influence on infectious diseases: it has been predicted higher vector and pathogen proliferation and reproduction rates at higher temperatures, extended transmission season, changes in ecological balances, and climate-related migration of vectors, reservoir hosts, or human populations (Lafferty 2009, Confalonieri 2007, Semenza and Menne 2007). If European countries get wetter and experience warmer temperatures in the winter, this could lead to a shift of climate suitability for *Ae. albopictus* to the northen regions (Caminade *et al.* 2012, Roiz *et al.* 2011).

VBDs are among the most well-studied diseases associated with climate change, due to their widespread occurrence and sensitivity to climatic factors (Confalonieri, 2007).

A combination of climatic and environmental factors such as temperature, humidity, rainfall and photoperiods influences the survival and reproduction rates of the vectors, affecting habitat suitability, distribution, and abundance and it may also change the intensity and temporal pattern of vector activity (particularly biting rates) throughout the year, as well as rates of development, survival and reproduction of pathogens within vectors (Confalonieri 2007, Semenza and Menne 2009, Rogers 2006).

In general, climate change is assumed to enable these potentially vector species to shift its spatial distribution to regions where they previously could not survive or establish (Walther *et al.* 2009). Temperate countries are already having a decrease in cold days and nights, and there is some evidence of climate-change related shifts in the distribution of tick vectors of disease, of some mosquito vectors in Europe and North America, and in the phenology of bird reservoirs of pathogens (Semenza and Menne 2009).

Climate change, combined with the socioeconomic development factors such as urbanisation, land-use, migration, or globalization, may shift the distributional range and transmission of the infectious disease (Semenza *et al.* 2012). Changes in the distribution limits of vectors at higher latitudes and altitudes have already been observed in Europe (Semenza and Menne 2009, Roiz *et al.* 2011*)*, Confalonieri 2007).

Caminade *et al.* (2012) studied the suitability of future climate for *Ae. albopictus* in Europe for both the current climate and future climate projections. Distribution maps of *Ae. albopictus* based on climatic features were produced, using a set of steps within different modelling processes (Caminade *et al.* 2012).

In addition, the ECDC recently developed a handbook on climate change impact, showing vulnerability and adaptation assessment to assist members in this process (ECDC - 2012). Lately, experts worldwide have highlighted the importance of updating surveillance activities and extending information on the role of these changes in the emergence of arboviruses, as a cornerstone tool for public health activities for dealing with climate change threats (Semenza *et al.* 2012, Weaver and Reisen 2010, ECDC - 2012).

2.4. Invasions and invasive mosquitoes

Biological invasions are causing serious concerns among scientific community due to their impact on ecological integrity, normally associated with homogenization and reduction of global biodiversity (Roy *et al*. 2012, Falk-Petersen *et al*. 2006; Perrings *et al*. 2005).

The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN) published the "100 of the World's Worst Invasive Alien Species, A selection from the Global Invasive Species Database" (Lowe *et al*. 2000). The electronic version can be accessed at http://www.issg.org/database/species/reference_files/100English.pdf (last consulted in August 2012).

Recent literature highlights the controversial definitions of the term invasive, which makes international regulation more difficult. An invasive organism can be considered as one (introduced intentionally or unintentionally by humans) which has established and is expanding its range in an area where it previously did not occur, this phenomenon has an intrinsic effect on the native community (Falk-Petersen *et al*. 2006).

The increase of the world trade system has resulted in a sharp growth in the number of new species being introduced to ecosystems and also the frequency with which such introductions are made (Perrings et al 2005). Mosquitoes are part of a small proportion of those introduced species that turn out to be harmful, historically causing substantial damage and control costs (Lounibus 2002, McNeely 2001).

Vector mosquitoes may cause relevant adverse effects on humans and vertebrates, with an impact on native species and the environment. Effects of competition and predation can affect native species, an invasive mosquito that replaces a resident species via competition or apparent competition may alter disease transmission (Juliano and Lounibos 2005).

The ability of laying desiccation-resistant eggs is an essential characteristic that commonly appears in invasive mosquitoes; it can help successful transportation through international trade of used tires or aquatic plants. This and the behaviour characteristic of colonizing larval habitats dominated by humans may increase the possibilities of invasion. Furthermore, superior interspecific competition including depletion of shared resources, chemical, physical, or mating interference can help the process to become invasive, but those characteristics are not essential. The mere capacity of occupying empty niches may facilitate the invasion of a new ecosystem (Juliano and Lounibos 2005).

These invasions are typically caused by repeatedly travelled routes and commerce. Besides, vector range expansions into a suitable environment are frequently followed by the emerging arbovirosis they can transmit. (Medlock *et al.* 2012, Weaver and Reisen 2010). *Aedes albopictus* is one well studied invasive species for which multiple hypotheses concerning the ecological processes operating during its invasions have been tested in several locations. However, the ecological processes that predominate in its interactions with other species are still not well known (Juliano and Lounibos 2005).

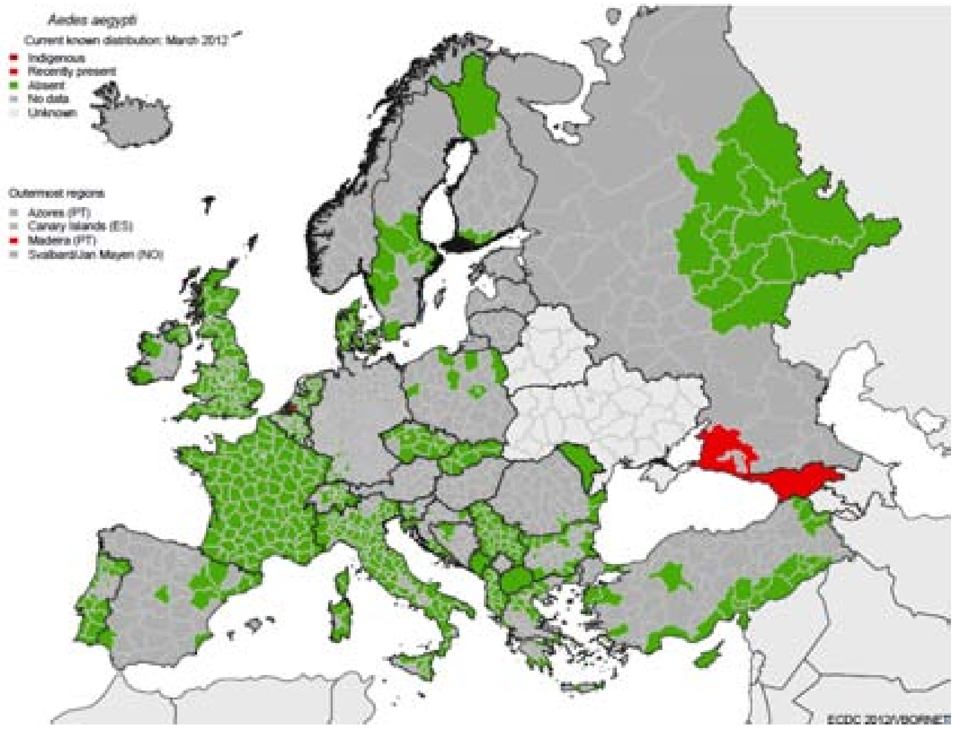
In Europe, as well as *Ae. albopictus*, other species of invasive mosquitoes are worrying the Health Authorities. Important species like *Aedes aegypti*, *Aedes atropalpus*, *Aedes japonicus* and *Aedes Koreicus* and *Aedes triseriatus* are monitored by VBORNET. Therefore every 3 months VBORNET generates updated maps of the current distribution status of the exotic mosquito species in Europe, based on a compilation of existing data from various sources, provided and shared by the members of the network. This unique map resource is accessible to the public (www.vbornet.eu) (last consulted August 2012).

Aedes aegypti (*Ae. aegypti*) could potentially have an impact on European public health. Known as the principal vector of DEN virus (Gubler 2002), this invasive species previously colonized Europe up to beginning of the 20th century, being the vector of DEN and YF (Reiter 2010). It is found currently in tropical and subtropical regions of the Americas, Africa and Asia, as well in the south eastern United States. In Texas it was the vector in some outbreaks of DEN in recent decades (Reiter 2001). *Ae. aegypti* is also endemic in the Indian Ocean Islands and northern Australia (Soumahoro *et al*. 2010). It has recently re-established itself in

Europe, Madeira Island (Almeida *et al.* 2007). It was detected but did not establish in Netherlands in 2010, (Sholte *et al.* 2010). The species is a highly effective vector of DHF (Wichmann and Jelineck 2003), YFV (Monath 1988), CHIKV (Gould and Higgs 2009) and epidemics of YFV.

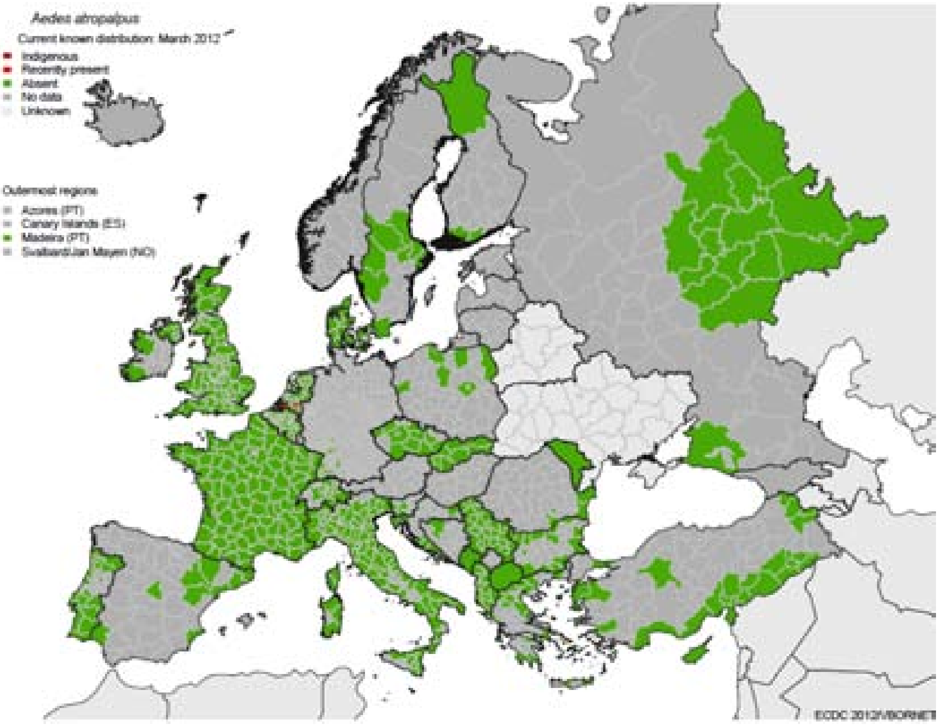
The current distribution of the main *Aedes spp*. detected in Europe is depicted on Figures 1, 7, 8 and 9. The overview of the vector status of the exotic aedine mosquito species intercepted or established in Europe is depicted in Table 3.

Figure 7 - Known distribution of *Aedes aegypti* in Europe.



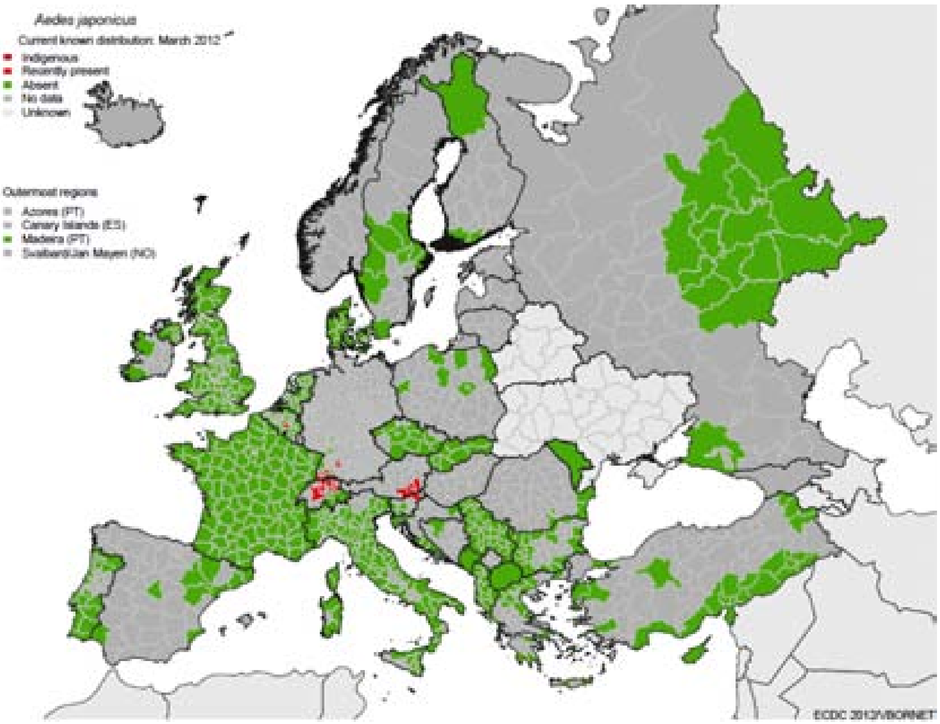
Source: ECDC Vebornet - based on field observations from the ECDC/VBORNET project (December 2011).

Figure 8 - Known distribution of *Aedes atropalpus* in Europe.



Source: ECDC Vebornet - based on field observations from the ECDC/VBORNET project (December 2011).

Figure 9 - Known distribution of *Aedes Japonicus* in Europe.



Source: ECDC Vebornet - based on field observations from the ECDC/VBORNET project (December 2011).

Table 3 - Overview of the Vector Status of the Exotic Aedine Mosquito species Intercept or Established in Europe (including *Ae. albopictus* previously shown on Table 2).

Pathogen			*aegypty*	*albopictus*	*atropalpu*	*japonicus*	*koreicus*	*triseriatus*
Virosis	*Alphavirus*	Chikungunya	■	■				
		Eastern Equine encephalitis		▨		░		░
		La Crosse		▨	░	░		■
		Venezuelan Equine encephalitis		▨				░
		Western equine encephalitis						░
	Flavivirus	Dengue	■	■				░
		Japanese encephalitis		▨		░		
		St Louis encephalitis				░		░
		West Nile		▨	▨	▨		▨
		Yellow Fever	■					░
		Zica	▨					
	Bunyavirus	Jamestown Canyon						▨
Nematodes	*Dirofilaria*	D. immitis i D. repens		■			░	

- ■ Proven vector in the field
- ▨ Found infected in the field and laboratory competence studies having potential role as vector, but not proven vector in the field
- ░ Only laboratory competences studies having showed potential involvement in transmission
- □ No vector or not known

Source: Medlock *et al.* 2012.

In Europe, several introductions of these invasive mosquito species have been intercepted during the last decades (ECDC 2012 – Guidelines for the surveillance of invasive mosquitoes in Europe – 2012):

- *Aedes aegypti* (*Ae. aegypti*): Russia 2001, Portugal-Madeira 2004, Abkhazia and Georgia 2007, Netherlands 2010 (limited local distribution)
- *Aedes atropalpus*: Italy 1996 and France 2003 (eliminated), Netherlands 2009 (limited local distribution)
- *Aedes japonicus*: France 2000 (eliminated), Belgium 2002 (localised), Switzerland and Germany 2008, Austria and Slovenia 2011
- Ae. *koreicus* (yellow dot): Belgium 2008 (limited local distribution), Italy 2011.
- *Ae. triseriatus* France 2004 (intercepted).

2.5. *Ae. aegypti* versus *Ae. albopictus*

Toward the end of the 19th century, the arrival of *Ae. aegypti* in Asia and the increase of its abundance in many cities (Tabachnick 1991) was accompanied by a decrease of the abundance of the native *Ae. albopictus* (Chan *et al.* 1971, Ho *et al.* 1972, Hawley 1988). In the opposite way, when *Ae. albopictus* recently established and spread in the United States (Sprenger and Wuithiranyagool 1986, Moore 1999), there was simultaneously detected a decrease in the range and abundance of *Ae. aegypti,* established in the Americas for centuries. *Ae. albopictus* has largely displaced *Ae. aegypti* and became the most abundant mosquito in artificial containers in most of the south-eastern United States (Lounibos 2002, Moore 1999, O'Meara *et al*. 1995). It was observed in areas where both species co-exist that they often share the same larval habitats (Simard *et al*. 2005).

Because of its physiological plasticity *Ae. albopictus* has been detected much further north than *Ae. aegypti* (Nawrocki and Hawley 1987). One major difference between these species is that *Ae. albopictus* has the ability to adapt to cold temperatures by ovipositing cold-dormant eggs that survive the winter of temperate regions (Mitchell 1995, Hawley 1988). The ability of the *Ae. albopictus* eggs to resist cold temperatures is likely linked to the ability to synthesize a high amount of lipids and to produce larger amounts of yolk lipid in cold temperatures. The larval lipogenesis of *Ae. albopictus* was found to be far more efficient than that of *Ae. aegypti* (Briegel and Timmermann2001) which partially explains the ability of tiger mosquito to spread further north (Paupy *et al.* 2009). Even in the case of tropical *Ae. albopictus* larval populations (nondiapausing) like the ones from La

Réunion, it is known they have a higher cold tolerance and larval survival when compared with *Ae. aegypti*. (Chang *et al.* 2007).

As said above, it was long believed that *Ae. albopictus* was a less efficient vector of arboviruses than *Ae. aegypti*. In the case of dengue transmission, it was considered an inefficient vector because it is not as well adapted to urban domestic environments and is less anthropophilic than *Ae aegypti* (Rezza 2012). However, field studies carried out in the USA, and laboratory experiments using conditions similar to the field studies have suggested that *Ae. albopictus* is favoured in competitive interactions with *Ae. aegypti* due to its better population growth rate (Juliano *et al.* 2004, Braks *et al.* 2004, Barrera *et al.* 1996). Indeed, the insect is becoming more important in causing arbovirus outbreaks as a consequence of a rapid widespread distribution (Paupy *et al.* 2010, Rezza 2012). Both species have become closely associated with the peridomestic environment (Reiter 2010).

2.6. Developing risk management strategies

One of the most striking consequences of globalization and climate change is the increase in the problem of invasive species. Current policies for managing native and invasive species are unlikely to be adequate in an era of rapid global change (Shirey and Lamberti, 2010).

Nowadays the invasive species Asian tiger mosquito is established in several countries of the Mediterranean basin affecting seriously the quality of life of its citizens because of the discomfort of its stings (Carrieri *et al.* 2008, *Curco et al*. 2008). There is increasingly serious concern because of the species vector competence, tiger mosquito has already transmitted CHIK and DEN in Europe (Paupy *et al.* 2009, Vazeille *et al.* 2008).

In the affected area of Catalonia the insect is widely established and there is a risk that it may continue to expand its geographic distribution. Thus, operational issues may be implemented by professionals, including field surveillance, methods of identification, key and optional procedures for field collection of population parameters, pathogen screening, and environmental parameters.

In areas where invasive mosquito species have become established, surveillance of their abundance and further spread is needed for timely risk assessment of pathogen transmission to humans, as well as control programmes. In the following chapter the main surveillance and control strategies will be detailed.

Chapter 3.

Managing the risk

3.1. Historical background

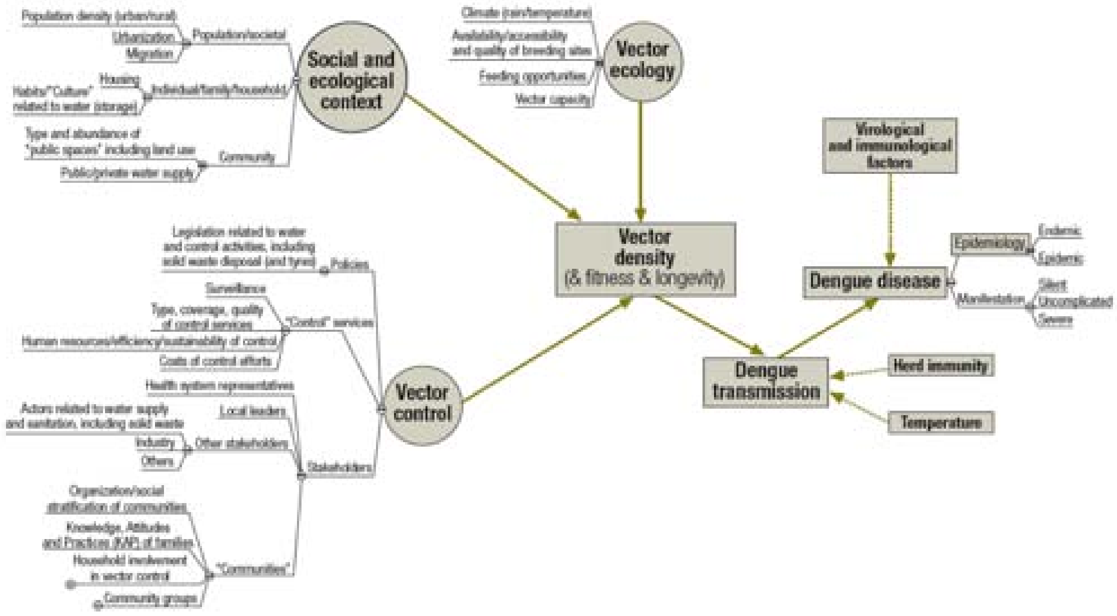
With the proven vulnerability of the Mediterranean region in relation to the expansion of the Asian tiger mosquito and its medical implications (Vazeille *et al.* 2008), environmental and health authorities in Europe have begun to coordinate themselves to manage the risk, which is still considered a challenging task (Enserink 2008).

When aiming at risk management, the interaction among multiple disciplines has proven to be important in studies performed in other continents (Arunachalam *et al.* 2010, Sanchez *et al.* 2009, Khun and Mandenson 2007). Health and environmental authorities should be called on to participate to the decision-making process, including for priority setting (Braks *et al.* 2011).

Surveillance and interventions are two issues considered as crucial for the implementation of early Vector-borne diseases (VBDs) detection systems. Parameters for vector and pathogen monitoring are highly desirable in Europe, as well as the precision and standardization of the available methods. Vector density (presence/absence), spatial and temporal variation in vector abundance and pathogen prevalence in vector population are the keystone parameters, which can only be assessed by active vector surveillance (Braks *et al*. 2011). Environmental risks, including social and ecological contexts are also part of the framework for vector and VBDs control tools (Arunachalam *et al.* 2010, WHO 2009).

A conceptual framework integrating different approaches to dengue control was developed in a recent study carried out in 6 Asian cities (Figure 10), where the main factors that may should influence dengue vector densities and ultimately viral transmission were analysed (Arunachalam *et al.* 2010).

Figure 10 –Ecological-Biological-Social (Eco-bio-social) determinants in dengue vector research.



Source: Arunachalam *et al.* 2010

Interdisciplinary involvement is indispensable to achieve control of a problem caused by a complex interaction of factors (Arunachalam *et al*. 2010, Sanchez *et al.* 2009, Khun and Mandenson 2007). Predictive models for a better understanding of the effect of globalization and climate change may be included in the general context, as these factors may influence the expansion of the spread of dengue to uninfected zones including Europe (Caminade *et al.* 2012, Semenza *et al.* 2012).

Several studies of vectors focus on the biological and behavioural characteristics of the insect (Roiz *et al.* 2010, Reiter 2007, Delatte *et al.* 2009-2008), the efficacy and cost of specific intervention (Griffin and Knight 2012, Esu *et al.* 2010, Kroeger *et al.* 2006), and also different strategies for vector management (Ballenger-Browning and Elder 2009, Erlanger *et al*. 2008). It is known that Integrated Vector Management (IVM) methods can reduce vector densities considerably (Horstick *et al.* 2010).

The biological and behavioural characteristics of the vector, the methods of surveillance and the strategies of control, including IVM are reviewed in this chapter.

3.2. Biological and behavioural characteristics of *Ae. albopictus*.

This mosquito was first described as the banded mosquito of Bengal by Skuse (1894) from specimens collected in Calcutta (India), however, due to the loss of the original specimens its neotype was first described by Huang (1968). The species belongs to the order *Diptera*, suborder *Nematocera*, *Culicidae* family, genus *Aedes*, subgenus *Stegomyia*, scutellaris group and subgroup *albopictus* (Hawley, 1988).

Reinert *et al*. (2004) recently revised taxonomy of the tribe *Aedini*, proposing the name change of the albopicta Stegomyia species. However, in this work we chose to keep the traditional name of *Ae. albopictus*. Although tropical forests are considered to be its original habitat, the species has developed the capacity to exploit artificial environments similarly of *Ae. aegypti*. As seen above, unlike *Ae. aegypti*, *Ae. albopictus* is capable of colonizing temperate areas permanently, by overwintering in the egg stage through a diapausing embryo mechanism induced by a short photoperiod experienced by the female (Hawley *et al.* 1989).

All stages except the egg have mobility. Female tiger mosquitoes may lay their eggs a little above the surface of water, on the wall of the receptacle. The length of the period of embryonic development depends mainly on the temperature and relative humidity to which the eggs are exposed, being periods from two to four days with temperatures between 24 and 27ºC (Hawley, 1988), relatively humidity of 70-80% and photoperiod of 16L/8D hours for 6 to seven days (Estrada Franco and Craig 1995, Hanson and Craig 1994).

Figure 11 – Female *Aedes albopictus* after a blood meal.

Figure 12 – Female *Ae. albopictus* after the sting © Roger Eritja
The abdomen appears red due to the ingested blood

Figure 12 – Eggs of *Aedes albopictus.*

Figure 12 – Eggs adhered to wooden surface may hatch when immersed in water, where larvae and pupa will be developed © Roger Eritja

Figure 13 – *Aedes albopictus* larvae.

Figure 13 – Larvae emerge to the surface, breathing through respiratory siphon © Roger Eritja

Figure 14 – *Aedes albopictus* pupae and *larvae.*

Figure 14 – Pupae of *Ae. albopictus* are active, short lived and do not feed © Roger Eritja

Figure 15 – *Aedes albopictus* pupae.

Figure 15 – An up close view of *Ae. albopictus* pupae © Roger Eritja

Figure 16 – Adult *Aedes albopictus* emerging from pupa stage.

Figure 16 – After emerging, the adult mosquito may rest allowing the hardening of the exoskeleton and wings © Roger Eritja

There are four larval instars, becoming larger after each moult. *Ae. albopictus* is often abundant in the peridomestic environment, particularly in areas with plentiful vegetation (Ellis 2001). During the larval and pupal stage, mosquitoes remain in the water; both these stages are adapted for swimming. The larvae of most species can be seen resting at the surface, hanging from the surface film by their respiratory siphon. The pupa does not feed during this transitional stage of development, as they transform from larvae to adults. When mature, the skin of the pupa splits open along its back and the fresh, adult mosquito slowly emerges into the air. After resting on the surface of the water for a few minutes, the adult mosquito usually moves to a sheltered spot close by to allow its outer skeleton and wings to harden. (Ellis 2001). Illustrations of *Aedes albopictus* are shown in Figures 11-16.

Larval stages of tiger mosquito are able to grow in different types and sizes of containers (Hawley, 1988). Some examples of immature habitat are depicted in figure 17 (a-d). Larvae of this species have been found among a variety of containers, bamboo poles, used tires, plant axils (Bromeliaceae), drums, troughs, vases, cans, plastic sheeting, small ponds, scuppers, coconut shells, clay pots, barrels, plant saucers and cubes are among the natural and artificial containers in which larvae of this species have been found. Usually they are found in clean water (pH 5,2- 7,6) (Roiz *et al*. 2007-b)

The population bionomics at larval and adults stages of *Ae. albopictus* are directly affected by temperature variations. In the literature, very different longevity values are available for populations from different regions of the world (Dellate *et al.* 2009, Monteiro *et al.* 2007). At 25ºC several studies showed similar results for the number of female eggs per female, ranging from 150 to 175 (Delatte *et al.* 2009, Braks *et al.* 2006, Gubler 1970, 1971).

At 30º C larvae can have a development of 6 days, 9 days at 25º C and 13 days at 20º C (Estrada-Franco and Craig, 1995, Lactin *et al.* 1995). The pupal stage lasts about 32-36 hours in males and in females 49-52 hours, with a minimum of 12 hours for the development of females (Hawley 1988).

Figure 17 a-d – Habitats of immature stages of *Aedes albopictus.*

Figure 17 a – Pool out of use
Source: Own elaboration

Figure 17 b – Drums
Source: Own elaboration

Figure 17 c – Receptacles with rain water
Source: Own elaboration

Figure 17 d – Catch basin
Source: Own elaboration

In a recent study carried out in the Indian Ocean island of La Réunion, average longevity results for females and males adult populations tested a 25ºC were 30 and 18 days, respectively. Maximum population growth was found to be when the temperature was between 25 and 30ºC, this range is suggested to be the most suitable for the development of the species. Adult highest survival rates were found at 15ºC and the lowest at 35ºC. The minimum development threshold temperature was estimated at 10.4ºC and the maximum at 40ºC; however, if no development was recorded at T<10.4ºC, 4.4% of egg hatching was observed at 5ºC (Dellate *et al.* 2009). Other studies carried out in Asia and North America have estimated this threshold value for adult season around 11ºC (Kobayashi *et al*. 2002, Hawley 1987).

Despite not having many references available on demographic parameters for *Ae. albopictus* in Europe, a study carried out in northern Italy estimated the lower temperature threshold for seasonal emergence of host seek female ranging from 12.6º and 13.5ºC, and for the end of the season ranging from 8.52ºC to 9.5ºC (Roiz *et al*. 2010). Also a previous study carried out in Rome estimated this threshold value around 11ºC (Toma *et al*. 2003).

Host feeding patterns of *Aedes albopictus* and *Culex pipiens* were recently studied in Barcelona urban zones and it was detected that all the blood ingested by the invasive mosquito was originating from humans (Muñoz *et al*. 2011). Normally reported as a generalist (Valerio *et al*. 2010, Kim *et al.* 2009 and Richards *et al.* 2006), its preference for a human blood meal had also been previously identified in Thailand (Harrington 2005).
The knowledge of demographic parameters such as densities, longevity, host preferences and duration of gonotrophic cycle are important to optimize the implementation of control methods. Besides, combined with research on vector competence, knowing these parameters is crucial to studying the success or viability of vector to VBDs in an area (Delatte *et al*. 2009-2008).

3.3. Surveillance methods

Entomological surveillance is an important tool to monitor vector populations and to identify areas with high density of infestation. It is used to determine changes in the geographical distribution and density of the vector and to evaluate control programmes and obtain indicators of the vector population over time. Thus it facilitates appropriate and timely decisions regarding interventions (Braks *et al*. 2011).

Sampling and trapping devices for arthropod of pathogens are important tools for the collection of ecological and behavioural data such as population densities, daily and seasonal abundance, vector distribution, and survival after control measures. These data are crucial in understanding the epidemic potential and in setting up early and effective control strategies (ECDC - Guidelines for the surveillance of invasive mosquitoes in Europe - 2012, Morrison *et al.* 2008, Ooi *et al.* 2006).

Numerous tools for monitoring the presence and/or following population densities of mosquitoes are commercially available, the best system chosen depends on the objectives, the level of infestation, available funding, and the skill of personnel. Once a given system has been adopted for monitoring and found satisfactory, it is advisable to maintain it (ECDC - Guidelines for the surveillance of invasive mosquitoes in Europe - 2012).

The main tools to monitor population densities are ovitraps, sticky traps, adult traps, human landing rates and larval and/or pupal counts in sentinel breeding sites.

The traditional larval indices are the Breteau index (number of positive containers per 100 houses inspected), the container index (the percentage of water-holding containers infested with larvae or pupae), and the house index (percentage of houses infested with larvae and pupae) based on the absence or presence of aquatic stages. These indices are used as epidemiologic indicators of dengue transmission in endemic regions, mainly to estimate the efficacy of interventions (Erlager *et al*. 2008; Esu *et al*. 2010; Silver 2008, Focks 2003). However, they are time consuming and barely cost-effective in countries where labour costs are high.

The fact that high-cost and long-term programs to control dengue vector population very often do not meet with success suggests that preventing and controlling the disease will require new vector surveillance and control technologies based on field studies of population biology and spatial-temporal distribution of the vector in its natural environment (Regis *et al.* 2008, Focks 2003). Besides, more recent analyses have shown that the Breteau index is not strongly related to dengue infection prevalence (Chadee *et al.* 2005); also larval abundance is a poor measure of entomological risk, whereas the number of female *Ae. aegypti* adults per person can be a risk factor for dengue infection (Rodriguez-Figueroa *et al*. 1995).

Table 4 - Entomological sampling methods – summary.

Type of monitoring methods (reference)	Recommended Application	Observations
Ovitraps (Fay and Eliason, 1966)	- Presence and absence of Aedes spp - Impact of various types of control measures (source reduction and insecticides)	- It is advisable to add some Bti in the water - The reliability in terms of quantitative estimation of the adult population density is controversial (Zhang and Lei, 2008, Focks 2003).
Lethal ovitraps (Ritchie *et al.* 2008, Zeichner and Perich 1999)	Similar to ovitraps with paddles substituted for insecticide-treated strip.	Increases the sensitivity of the monitoring with ovitraps.
Sticky traps (Ordonez-Gonzalez *et al.* 2001)	- Identification of the species - Recapture marked *Aedes* spp. in dispersal studies	- It needs to be replaced frequently, yard inspections are labour intensive - Other non-target insects may also stick in the glue
CDC backpack Aspirator (Clark *et al*. 1994)	- To monitor host-seeking and overwintering females - To identify household risk factors for dengue infection	- Provides information about the biology and behaviour of the species - When using light many other insects are trapped.
BG Sentinel trap (Geier *et al.* 2006, Kroeckel *et al*. 2006)	- To monitor host-seeking females	A mosquito collection device that incorporates visual, anemotactic and olfatory attractants.
Magnet trap (ECDC – 2012*, Kline 2002)	Used to protect people outdoors. Useful for long term sampling.	- Mosquitoes killed by electrocution - Attracts mosquitoes within a radius of 10 m. It is heavy and expensive.
Human Land Collection (ECDC - 2012*)	- To study the diet periodicity of Aedes attraction to host. - Sensitive for monitoring adult *Ae. aegypti* abundance.	-Ethical regulations must be taken into account - Location and time must be chosen carefully
Traditional Larval Indices (HI, CI and BI) (Silver 2008, Focks 2003)	To monitor vector control progress, to determine if prophylactic levels have been achieved.	The indices have limited use in accessing adult abundance and or dengue transmission risk.
Larval Survey Strickman and Kittayapong (2002)	To estimate abundance of *Aedes* larva in each container, and to identify species.	An estimate of the infestation level is obtained, which is a benefit compared with traditional indices.
Pupal/demographic survey. (Nathan and Knudsen 1991)	Estimation of mosquito borne disease transmission threshold	Because pupal mortality is slight and well-characterized, the number of pupae is highly correlated with the number of adults

* ECDC – Guidelines for the surveillance of invasive mosquitoes in Europe-2012

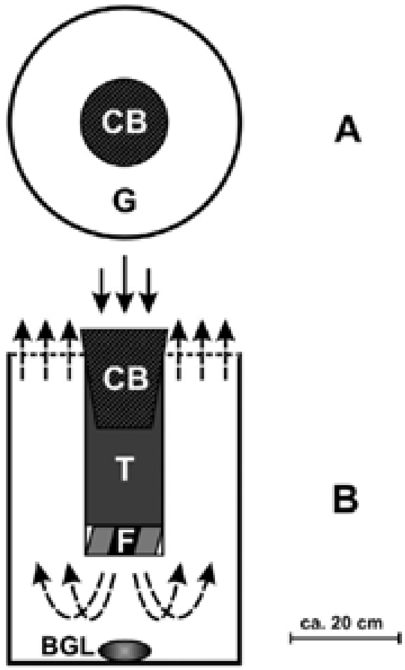
Some authors suggested the pupal demographic surveys, where all foci with pupae are identified and pupae counted or estimated, as the most promising method for epidemiological studies because a strong correlation has been shown between number of pupae and mosquito density (Chadee *et al*. 2007a,b; Nathan *et al.* 2006; Strickman and Kittayapong 2003; Focks 2000). However, in practice the challenge of finding all foci (for example, in catchment basins for the drainage of rainwater) can be a difficult issue, consuming a large amount of work time. Also the presence of pupae in a container is discontinuous, highly dependent on timing (Carrieri *et al*. 2012, Nathan *et al*. 2006). The main monitoring methods are summarized on Table 4.

Given the biological specificities of *Aedes spp.* populations, trapping eggs may be a more appropriate method of locating sites and times where action should be concentrated to prevent or lessen the intensity of disease outbreaks. Using mosquito ovitraps for vector surveillance seems to be a current trend in dengue endemic countries, since this method allows better assessment of infestation densities than the conventionally used methods based on the search for larvae (Ai-leen & Song 2000, Braga *et al.* 2000, Polson *et al.* 2002, Morato *et al*. 2005).

The adult trap BGSentinel (Biogents GmbH, Regensburg, Germany) has been shown to work particularly well in collecting disease vectors and nuisance mosquito species from around the world. BGS-traps consist of a folding white bucket with a cover made of gauze. In the middle of the cover there is a black tube where a fan working with electricity (12 V) is able to suck inside the mosquitoes which near the tube. Mosquitoes come close to the trap lured by the attractant spread by the fan, BGLure (BioGents GmbH) is a combination of lactic acid, ammonia, and caproic acid, that have been identified from human skin (Krockel *et al.* 2006). A schematic of the trap is depicted in Figure 18.

Studies carried out in Australia, USA and Brazil show that the BGS-trap may be an effective tool for collecting *Ae. aegypti* (Krockel *et al.* 2006, Maciel de Feitas *et al.* 2006, Rose *et al.* 2006, Williams *et al.* 2006, 2007) and *Ae. albopictus* (Ritchie *et al.* 2006). The device has been shown to attract male and gravid or bloodfed female *Ae. albopictus*. This trap was suggested as an invaluable addition to mosquito surveillance and control programs, especially to those interested in furthering their understanding of the role *Ae. albopictus* plays in arbovirus transmission. (Ritchie *et al.* 2006).

Figure 18 - Functional diagram of the BG Sentinel –trap.



(A) – View from above
(CB) – Catch bag
(G) – White gauze cover
(B) – Longitudinal section of the trap
(T) – Black tub
(F) – Fan
(BGL) – BG – Lure.
Arrows indicate the direction of the airflow.
The blend is constantly emitted in a fixed ratio from long-lasting dispenser.

Source: Kröckel *et al.* 2006

In addition, there is another version of CDC-trap, known as Miniature CDC. The devices works without light for the capture of host-seeking females. However, it is not very efficient for invasive mosquito species and also needs a source of carbon dioxide (ECDC – Guidelines for the surveillance of invasive mosquitoes in Europe – 2012).

Surveillance of mosquitoes population is normally an intervention carried out by the national or regional government (supra municipal) with previously defined methodology to obtain comparable results (Albieri *et al.* 2010, ECDC – Guidelines for the surveillance of invasive mosquitoes in Europe – 2012). Municipal technicians must frequently access the data obtained to take the appropriate decisions about control. Using ovitraps in combination with BGS-traps to monitor mosquito populations in countries with mosquito-infested areas is recommended. (ECDC – Guidelines for the surveillance of invasive mosquitoes in Europe – 2012).

Some behavioural characteristics of invasive mosquitoes may help to determine the suitable places to install mosquito traps. Shaded areas below three meters high, protected by the wind are recommended, as it is known that mosquitoes prefer to fly relatively close to the ground and also uses "shrub-corridors" (e.g. hedges) to go from one place to another, rather than crossing open terrain (ECDC – Guidelines for the surveillance of invasive mosquitoes in Europe – 2012). Ovitrap and BGS-trap densities applied for different studies are listed in Table 5.

Table 5 – Examples of trapping: objective, trap density and reference.

Trapping objective	Trap density	Reference
To measure the dispersal of Aedes spp fed on blood with rubidium chloride (RbCl) (mark-release-recapture MRR experiment)	3.2 a 7.3 ovitraps/ha	Honorio *et al.* 2003
To access the spatial dispersion	0.05-0.5 ovitraps/ha	Angelini. 2006
To determine the discomfort	0.09 ovitraps/ha	Angelini 2006
To estimate the effectiveness of campaigns at territorial level	0.005-0.02 ovitraps/ha	Angelini. 2006
Determine sample size (Number of BGS/area)	6,67 BGS trap/ha	Williams *et al.* 2007
To evaluate the nuisance level	0.125 ovitraps/ha	Carrieri *et al.* 2008
To access the spatial dispersion	0.024 ovitraps/ha	Roiz *et al.* 2008
Mosquito population surveillance and control	0.63- 1.29 traps/ha	Regis *et al.* 2008
Mark-release-recapture MRR experiment		David *et al.* 2009
Compare mosquito abundance in different study areas	0.0625 BGS traps/ha	Unlu *et al.* 2011
Mosquito population surveillance and control. Determination of sample size (Number of ovitraps) Determination of the epidemic risk threshold	0.0261 ovitraps/ha	Albieri *et al.* 2010 Carrieri *et al.* 2011 Carrieri *et al.* 2012
Adult mosquito surveillance	0.2 BGS trap/ ha	Tan *et al.* 2011
To assess the distribution and population abundance of *Ae. albopictus*	0.02 ovitraps/ha	Giatropoulos *et al.* 2012
Mosquito population surveillance and control	0.05 ovitraps/ha	Marche region (Italy) surveillance 2007*

*Source: http://www.saluter.it/documentazione/rapporti/contributi/contributi-n.-50-2

The optimal number of traps to be placed in an urban area depends on different factors, like the species density and dispersion (Albieri *et al.* 2010) and may also vary according to the objective of their application and the desired precision level (Carrieri *et al.* 2011a, Albieri *et al.* 2010, Williams *et al.* 2006). High levels of precision require a large sampling effort at low population densities. If an outbreak occurs, it is necessary to reinforce the surveillance system, and the number of traps should be increased (ECDC – Guidelines for the surveillance of invasive mosquitoes in Europe – 2012).

Lack of information about the costs of monitoring systems used to be a limiting factor, but in the northeast Italian region of Emilia Romagna where an outbreak of CHIK virus occurred in summer of 2007, the public Health Service has developed a homogeneous monitoring system for *Ae. albopictus* to obtain information about its temporal evolution (Angelini 2006) with the estimated global cost considered to be 7 € per ovitrap and 26 collection turns (Carrieri *et al.* 2011b), with the routine management of field ovitraps conducted by municipal technicians.

The recently published ECDC guidelines recommend mosquito collection methods according to the aims of mosquito surveillance, with indications of density, frequency and period of the year. In addition, it provides cost estimates of the various activities associated with the main procedures (ECDC – Guidelines for the surveillance of invasive mosquitoes in Europe – 2012).

3.4. Vector control

3.4.1. The background of Vector control in Spain

Early studies on mosquitoes in Spain were published by the English physician Mc Donald, motivated by the presence of malaria (Mc Donald, 1900). During the early twentieth century, Pittaluga et al. conducted and published studies on the species complex *Anopheles* (Meigen, 1804) and its role in malaria transmission in Spain (Pittaluga *et al.* 1901-1903).

In 1912 the first complete list of Iberian species of mosquitoes was published, which listed 12 species in Spain including *Aedes aegypti* (Linnaeus, 1762), vector of dengue and yellow fever (Arias Encobet, 1912).

Various anti malaria services in the country set up entomological research studies which made important contributions to studying the malaria vector. (Bueno Marí 2010). Many publications were then produced, especially good were Gil Collado, 1930; Elvira, 1930; Buen, 1935 y Nájera, 1935. The list of Iberian culicoides increased to 30 species. (Encinas-Grandes, 1982).

With the disappearance of malaria in 1961, scientific and technical work on mosquitoes decreased drastically in the country (Bueno Marí 2010). Nevertheless other studies were conducted on the biology of the species and their role as vectors. Some key studies were published: Margalef, 1943; Torres Cañamares, 1945; Clavero, 1946; Rico-Avello y Rico, 1953; Romeo Viamonte, 1950; Gil Collado, 1954, Garcia Calder-Smith, 1965; Contreras Poza, 1971; Baez and Fernandez, 1980 The outstanding work of Antonio Encinas-Grandes (1982) extended the current knowledge base of mosquitoes, citing 56 species in Spain (Roiz *et al*. 2007, doctoral thesis).

In the beginning of the eighties came the first Mosquito Control Services (MCS), promoting investigations on Culicoides to combat the inconvenience of its hematofagic action in the human population. The MCS, supported by public funding, is staffed by professionals, which has allowed it to develop a strong vector control competence. (Ruiz and Cáceres 2004, Eritja and Aranda 1995, Aranda and Eritja 1992). Also detailed research was done into the biodiversity, ecology and phenology of the culoides present in the area of action. (Cáceres and Ruiz 2004, Eritja and Goula 1999, Padrós *et al*. 1993).

In recent decades, several studies related to the detections and biology of species have been published, including: Sánchez-Covisa, 1985; López Sánchez, 1989; Curto, 1990; Jorda *et al.,* 1993; Llave Correas and González Mora, 1996; Lucientes *et al*., 1998; Eritja, 1998; Aranda *et al*., 2000; Melero-Alcibar y Salom, 2002; Melero-Alcíbar, 2004, Escosa and Aranda, 2004; Ruiz and Cáceres, 2004; Check list of the mosquitoes of Spain Eritja *et al.*, 2000, Eritja *et al.,* 2005.

The entry of the invasive species *Aedes albopictus* in Catalonia and later in other regions of the Iberian Peninsula definitely pushed ahead research and technical work on mosquito control. Studies are published annually in different fields related to the species, which is now a challenge to global public health. Recent important publications include: Aranda *et al.* 2006, Giménez *et al.* 2007, Curcó *et al*. 2007, Roiz *et al.* 2007, 2008, Bueno Marí 2010; Muñoz *et al.* 2011, Vasquez 2011, among others.

Currently there are different MCS operating in the country to combat the discomfort caused by culicoides, the County Council's SCM Baix Llobregat, MCS Ebro Delta, Huelva County Council MCS, MCS Roses Bay and Lower Ter. To these must be added the MCS Monegros (Huesca) and the MCS of Maresme County Council (located along the Catalan Mediterranian cost), which became operational recently to cover an area of the Catalonian coast.

Within the European context, it is the association EMCA (European Mosquito Control Association), which has 185 members from 36 countries, including Spain which occupies a prominent place, with 13 other members.

3.4.2. Vector control methods

A. Public Health education

The availability of mosquito breeding spots in urban areas is closely related to certain human activities, outdoor water collection tanks or waste containers are some examples of this. Thus, control methods should be focusing on these factors. Educating affected human populations about mosquito habitats and encouraging them to reduce potential breeding sites in their households, as well as advising people to wear protective clothing and repellents can help to reduce mosquito nuisance and arbovirus transmission (Medlock *et al.* 2012).

Studies worldwide have detected that health promotion and education and community participation are perceived as crucial approaches for the success of vector control (Horstick *et al*. 2010, Baly 2009, Sanchez *et al*. 2009, Erlanger *et al.* 2008, Heintze *et al.* 2007, Khun and Manderson 2007, Perez *et al.* 2007). However, traditional passive means of public education alone may be not sufficient to motivate residents to reduce backyard mosquito-larval habitats (Bartlett-Healy *et al.* 2011).

Many actions can be carried out by a health education programme, such as distribution of printed recommendations, educational meetings, educational outreach visits, the involvement of local opinion leaders, the involvement of national institutions and the use of the mass media (radio, television, newspaper, leaflets, posters) for the implementation and improvement of vector control programmes (Heintze *et al.* 2007). The final intention of changing behaviour must aim at producing results that are visible and can be monitored (Ballenger-Browning and Elder 2009).

B - Source Reduction

Source reduction consists of reducing sites that could provide suitable aquatic habitats for larval development. Crucial to the efficacy of the educational campaign, larval source reduction has been and continues to be the key component of successful dengue vector programs around the world (Whelan *et al*. 2009; Gubler 1989). It is very important to inform the affected population about the elimination of water holding containers, which is basically a measure of self-protection, especially in the case of *Ae. albopictus* which has a flying range below 200 m. (Marini *et al,* 2010, Lacroix *et al.* 2009).

Educational outreach visits (door-to-door) can greatly affect the distribution of mosquito larvae in a neighbourhood, by limiting the number of available habitats for ovipositing mosquitoes (Richards *et al.* 2008). In the prevention of mosquito stings and mosquito-borne disease, the contribution concentrates primarily on the removal of breeding points in private properties. However, the vast number of breeding containers that *Ae. albopictus* can use as larval habitat and the problem of access to the private gardens can increase the difficulties of this method. The implementation even of such a simple program requires careful planning, and conscientious execution; to have a lasting effect it must be indefinitely sustained (Medlock *et al.* 2012).

In looking for an effective program, the local community will need to be continuously invited to collaborate with the control programs, and allow the civil workers to inspect their gardens. Householders should be well informed about the health threats of having larval habitats in the gardens, and be well motivated to take preventive measures in their dwellings and in the neighbourhood, for their own protection. Behavioural changes in relation to water use and storage are highly desirable, and may be promoted by health authorities. In addition, education programs must target behaviours that are feasible for the target population in terms of abilities, time and resources. (Medlock *et al.* 2012, Vanlerberghe *et al.* 2009, Ballenger-Browning and Elder 2009, Heintze *et al.* 2007).

C- Biological control

Biological control consists of the introduction of larvivorous organisms, defined as predators of the aquatic larval stage of mosquitoes in a water container (Griffin and Knight 2012). Many organisms such as fish, copepods and bacteria are predators of mosquitoes larvae (Kumar and Hwang 2006, Brodman and Dorton 2006). However, fish are the most common and widely studied type of predator used for biological control of mosquitoes. Enhancing larvivorous fish populations in mangroves has potential as an effective and often environmentally friendly mosquito control approach (Griffin and Knight 2012).

Also microbial larvicides, especially *Bacillus thuringiensis israelensis* de Barjac (Bti) are frequently used in *Aedes* control, acting as a stomach poison in the larva midgut. Applying Bti in a container, mosquito production can be reduced or eliminated, a high efficacy and selectivity has been shown, combined with its assumed safety for the environment, including non-target fauna, (Person Vinnersten *et al.* 2010). However, mosquito larvae are an important food sources for many birds (King and Wrubleski 1999), bats (Fukui *et al*. 2006), frogs (Vignes 1995) and other arthropods (Paetzold and Tockner 2005). In a recent study carried out in France significant effects of Bti spraying were observed on many reed-dwelling invertebrates serving as food to passerines, as well as on the diet and breeding success of house martins nesting in rural estates and small towns (Paolin *et al*. 2011). Additionally, lack of studies about residual toxicity in relation to Bti accumulation is a cause of concern (Tilquin 2008).

Copepods (Copepoda) show great promise as a tool for the biological control of container-inhabiting mosquitoes. In parts of Vietnam the use of the crustacean Mesocyclops was found to prevent dengue transmission (Kay and Nan 2005). Besides, in a recent study carried out on Florida Mesocyclops longisetus showed to be able to eliminate mosquito larvae for extended periods in container habitats of urban/suburban backyards, despite the fact that it may not be practical to introduce a sufficiently large number to the largest containers (Soumari and Cilek 2011). Simultaneous introduction of Bti and copepods could also be an option for biological control of container-inhabiting mosquitoes, as they can be mixed in the same tank (Soumari and Cilek 2011, Tietze *et al.* 1994, Marten *et al*. 1993).

Biological methods are considered to be well accepted by householders (Morrisson et al 2008), however, labour intensive maintenance and high turnover of organisms for containers with frequent use were considered the primary disadvantages of biological methods. Also seasonal changes may dry some temporary containers, resulting in the death or escape of the control organism (Ballenger-Browning *et al.* 2009).

The use of Bti would be more recommended as it is specific against mosquito larvae and it is considered environmentally friendly, however frequent re-treatment is necessary with the commonest formulations (WHO 2006), as it is usually active for one to two weeks at most (Lacey and Lacey 1990).

D- Chemical larvicide treatment

The use larvicides on permanent water holding containers has an effective action for a prolonged period of time and thus can contribute to systematically decrease the number of adults for a long period of time (Poletti *el al.* 2011). Biorational larvicide is preferred, this is defined as "any type of insecticide active against pest populations, but relatively innocuous to non-target organisms, and, therefore, non-disruptive to biological control" (Stansly *et al.* 1996).

Among the biorational larvicides used in immature control there are the larval growth regulators (IGRs) and the microbial larvicide (see C- Biological Control). Insect growth regulators have become more widely used in recent years.

Juvenoids, as methoprene and pyriproxyfen, interfere with transformation of the immature stage to the adult, while chitin synthesis inhibitors, such as diflubenzuron (DFB), triflumuron and novaluron, inhibit cuticle formation (WHO 2006).

DFB and pyriproxyfen offer an excellent potential for the control of the Asian tiger mosquito, they are frequently used in control programs (Carrieri *et al.* 2009; WHO 2009), because adverse effects on associated aquatic non-target organisms in the habitat of *Ae. albopictus*, such as small artificial containers would be of minimal concern (WHO 2006, Charmilot *et al*. 2001, Ali *et al.* 1995). However recent studies detected negative effects of the DFB on the survival of zooplankton, thus, disrupting the food chain balance (de Souza *et al.* 2011).

DFB is a direct-acting insecticide normally applied directly to water. It is used in public health applications against mosquito and noxious fly larvae. WHO considers DFB adequate for use as a mosquito larvicide in drinking-water in containers, particularly to control dengue fever. The recommended dosage of diflubenzuron in potable water in containers should not exceed 0.25 mg/litre under the WHO Pesticides Evaluation Scheme (WHO 2008).

IGRs and the microbial insecticides *Bacillus thuringiensis israelensis* (see section 3.4.2.C) are possible alternatives to common chemicals for larviciding. However, IGRs affect non-target organisms and should not be used in breeding sites with an abundance of arthropod species, unless an impact assessment has been carried out. Bti would be more feasible to be used for larvea control (see C- Biological Control), although it is active for a shorter period.

E- Insecticide spraying

After the Second World War, focal application of the synthetic pesticide dichlorodiphenyltrichloroethane (DDT) to infested containers and their surroundings was one of the first chemical control measures used to target adult stages of the dengue vector. Significant reductions in vector populations were achieved, according to the Pan American Health Organization; the invasive *Aedes aegypti* was eradicated from 22 countries in the Americas. However, the development of DDT resistance was one of the main factors that led to the re-emergence of dengue from the 1960s onward (WHO 1997).

Although second and third generation insecticides became available (e.g. malathion and pyrethroids) it is known that chemical control of dengue vectors has shortcomings, including environmental contamination, bioaccumulation of toxins and concerns regarding human toxicity, which are especially linked to the use of insecticides in drinking- water containers (Curtis and Lines, 2000, Erlanger *et al*. 2008).

One of the most common methods for controlling arthropod vectors, particularly mosquitoes, is the application of insecticides by either ground or aerial sprayers. Adult mosquitoes are easily controlled with insecticides applied at extremely low rates. Ultra Low Volume (ULV) mists involve the application of small quantity of concentrated liquid insecticide (Hoffman *et al.* 2009), specifically the minimum effective volume of the formulated product without any further dilution. Both ground and aerial ULV applications have been the standard method of mosquito adulticiding worldwide for more than 45 years (Bonds 2012).

Droplet size is the main factor that affects the efficiency of the ULV space sprays: It must be small enough to be produced in sufficient numbers for probability of contact, but also should be large enough to impact on the body surface of adult mosquitoes. Several studies agree that optimal droplet size should be less than 20 μm in diameter (Bonds 2012).

However, chemical control, particularly space spraying, has limited acceptability, partially explained by its perceived or real negative impacts on the environment and human health (Curtis and Lines 2000). *Ae. albopictus* is susceptible to pyrethroids, which have been largely used as adulticide. Alpha-cypermetrin, Lambda-cyhalothrin and Deltamethrin are authorized adulticides in the European Union (http://www.pesticideinfo.org/Detail_Country.jsp?Country=European%20Union) (last consulted August 2012) that have showed great potential for adult control (Harris *et al*. 2010; Pettit *et al.* 2010; Thanispong *et al.* 2008).

Application of adulticides may be restricted to high risk situations and should be done only by qualified professional users directly on the typical resting sites of *Ae. albopictus* female mosquitoes (WHO 1997):

- Heavy vegetation near their larval breeding sites or their hosts.
- Areas of high humidity along the shores of streams, rivers and lakes.
- Cool, moist places along roadsides (e.g., in culverts, under bridges, in catch-basins).
- In piles of firewood, lumber, or other stored materials.

Targeting adult vectors by outdoor ULV application of insecticides and indoor aerosol cans remain the methods of choice for emergency interventions during dengue outbreaks. However, the permanent adoption or rejection of the use of peridomestic spraying for vector control is still dependant on more research, and a clear guidelines for appropriate implementation and monitoring of its effect is not available yet (Esu *et al.* 2010).

F- Solid Waste Management

Solid Waste management is an important issue that affects the efficacy of mosquito control programs, being part of environmental management methods to control *Aedes* and reduce human vector contact (WHO 1997). In some African, Asian and Caribbean countries householders typically continue to store water because supplies are not reliable. Vector control efforts should promote waste management.

The elimination of plastic containers, used tyres and other receptacles are actions of critical importance to urban Aedes control. If storage tanks, drums and jars are required, they should be efficiently covered (WHO 1997). Advocacy is a process through which groups of stakeholders can be influenced to gain support for and reduce barriers to specific initiatives or programmes. Multiple strategies, often used simultaneously, are the key to the success of any advocacy effort. (Heitze *et al*. 2007, Ballenger-Browning and Elder 2009, Erlanger *et al*. 2008).

G- The sterile insect technique (SIT)

The Sterile Insect Technique (SIT) has been used successfully for suppressing or eliminating a number of agricultural pests (Dyck *et al.* 2005). The Release of Insects Carrying a Dominant Lethal (RIDL) system is a variant of SIT which replaces irradiation by genetically engineered inducible sterilization.

Currently the use of SIT is still under development with regard to mosquito control, however in recent years several studies and extensive research and development have been on-going for preparation of commercial scale SIT (IAEA 2008, Benedict and Robinson 2003) and encouraging results have been obtained in research (Boyer *et al.* 2011, Nolan *et al.* 2011, Phuc *et al.* 2007).

A recent study produced a modified male *Ae. aegypti* with a gene that limited wing development, thus after mating with females, the gene is passed on, resulting in offspring unable to fly. These results are ready to be used in large-scale mosquito control (Fu *et al*. 2010).

A mathematical model using RIDL male releases in SIT program developed in a recent study suggested that such a program may eliminate VBDs at lower cost than the direct and indirect costs saved from averting illness (Alphey *et al.* 2011).

Despite the need for more studies to be carried out to assess the effectiveness of this strategy on wild mosquito population, SIT is a new approach to mosquito control that can be part of a wider integrated pest management program involving different technologies (Medlock *et al.* 2012, Nolan *et al*. 2011).

H – Bacteria infection techniques

Some recent studies found out that it is possible to decrease transmission through mosquito infection with bacteria from the *Wolbachia* genus. Moreover, the bacteria are transmitted from one generation of mosquitoes to the next, making the infection self-sustaining. *Wolbachia* has been successfully introduced into *Aedes aegypti*, the main transmitter vector of dengue. Small trials of the infected vectors in Australia look promising, and show that the bacteria-laden insects quickly integrate into native populations. Within 14 weeks of the release of 250,000 *Wolbachia*-infected mosquitoes, 90% of the populations in the test areas were positive for the bacterium (Hoffman *et al.* 2009).

I – Insecticide-treated materials

Insecticide-treated bed nets aim to deter mosquitoes and kill them when they land. Although relatively inexpensive, they work when the person is underneath indoors, and the technique is susceptible to growing resistance. Also safe pesticides must be applied. Treated-bed nets as well as jar covers and treated curtains have been used mainly in endemic areas of malaria and also for other diseases like leishmaniasis and Chagas disease. Treated materials have also been implemented for dengue control in areas where *Aedes aegypti* is the vector, decreasing significantly the larval indices (Erlanger *et al.* 2008, Kroeger *et al.* 2006). However, the method may not be efficient for the *Aedes albopictus,* due to the exophilic "daytime biting" behaviour of the species.

J – Physical control

Various physical control methods have been developed either to reduce vector population densities. Physical control methods mainly consist in the use of oil surface films and Polystyrene Beads in the stagnant water for the control of breeding sites, which constitutes a simple, economical and long last effect (Becker *et al*. 2010). Such a layer interferes with mosquito oviposition and larval or pupal access to air.

3.5. Integrated Vector Management (IVM)

The WHO defined IVM as "a rational decision-making process for the optimal use of resources for vector control". Its goal is to make a significant contribution to the prevention and control of VBDs, responsible for 17% of the global burden of parasitic and infectious disease. Implementation of IVM requires institutional arrangements, regulatory frameworks, decision-making criteria, and procedures that can be applied at the lowest administrative level (WHO 2008).

The IVM programs are based on knowledge of the biology of pests and their relationship with their environment. Therefore, control strategies generally used as part of such programs include improved hygienic conditions, using methods of pest exclusion, manipulation of the habitat, awareness of the population to avoid behaviour that may contribute to the spread of the pest and, if necessary, the selection of specific pesticides with minimal toxicity and minimal impact on the environment (WHO 2008).

Over the past decades Integrated Vector Management (IVM) or Integrated Pest Management (IPM) programs that include surveillance, source reduction, larvicides and biological control as well as public education have been developed, usually facilitated through community-based approaches.

Although the ambitious goal of eradication of *Aedes spp*. does not seem to be reachable (Gubler 2001), the main components of IVM strategies are:
1- Surveillance, source reduction and education
2- Larviciding of water storage containers
3- Selective adulticiding (ultra-low-volume) when the adult mosquito density is too high in sensitive areas
4- Communication and education on vector prevention,
5- Enforcement of mosquito control legislation (Horstick *et al.* 2010, Kittayapong 2008, Heintze *et al.* 2007, Winch *et al.*2002).
There is growing evidence that when combined activities are implemented with a sustainable methodology they can be highly effective in reducing vector populations (Ballenger-Browning and Elder 2009, Erlanger *et al.* 2008, Heintze *et al.* 2007). This also requires decision-making skills that support intersectoral action and are able to establish vector control and health-based targets. Intersectoral collaboration among partners is a key component of IVM (WHO 2009).

Despite the IVM approach working well for control of the immature stages because there are many options to choose from, few options exist for use against adult mosquitoes. Besides adulticides, available options consist of personal protection and public education. (Kline 2006; Heintze *et al*. 2007).

In the Global strategic framework for IVM, the WHO invited member states to accelerate the development of national policies and strategies (WHO 2008). Also the use of new emerging technologies needs to be encouraged to develop effective IVM programs targeting mosquitoes (Erlanger *et al.* 2008).

3.6. Developing, implementing and testing an IVM

IVM is the combination of available control methods in the most effective, economical and environmentally friendly manner to maintain vector populations at acceptable levels. It has been implemented worldwide and in some cases with a significant reduction of larval indices and *Aedes spp*. densities (Arunachalam *et al.* 2012, Erlanger *et al*. 2008, WHO 2008). Knowing the biology and ecological behaviour, including feeding preferences of the species, may help to set up a control strategy optimizing the available resources.

The development of an efficient monitoring network is an essential tool for verifying the effectiveness of the control measures (ECDC - Guidelines for the surveillance of invasive mosquitoes in Europe - 2012, Carrieri *et al.* 2011a). Pupal survey technique, ovitraps and BGS-traps have been suggested lately as effective monitoring systems for temperate populations of *Ae. albopictus* (Carrieri *et al.* 2012, Unlu *et al.* 2011, Farajollahi *et al.* 2009, Krockel *et al.* 2006, Focks 2003).

Although the pupal demographic survey seems to be the most appropriate index for epidemiological studies, as it exploits the strong correlation between the number of pupae and the number of adults in a definite area (Chadee *et al*. 2007 a,b; Focks and Alexander 2006), as said before, its adoption as a monitoring method could be very costly and time consuming. Thus, ovitraps remain an economically affordable method to estimate the mosquito population density in a IVM strategy (Carrieri *et al.* 2011a).

In the design of a proactive IVM strategy, the creation of public awareness is crucial. To achieve the collaboration of the citizens, primarily for the removal of breeding containers on the dwellings, source reduction program may be implemented; the population may be informed about the prevention measures (Heintze *et al.* 2007).

As a complementary intervention, the use of biorational larvicide to control immature instars of the insect is recommended in IVM programs, as the number of catch basins on public places may affect the density and dispersion of *Ae. albopictus* in the urban environment. Thus, periodic larvicide treatment may be required for a control program (Carrieri *et al.* 2011a, Vanlerberghe *et al.* 2009).

Combining activities like source reduction, insecticide treatments and environment management towards an integrated strategy in mosquito control has been suggested as a sustainable methodology that can be highly effective (Erlanger *et al.* 2008, WHO 2008).

In the following chapter a case study of the implementation of combined interventions as an IVM strategy to reduce tiger mosquito population is presented, its effectiveness was assessed by the use of ovitraps.

Chapter 4
Case study: Managing the tiger mosquito in Sant Cugat

4.1. History

In the summer of 2004 the first introduction of populations of the tiger mosquito in the Iberian Peninsula was detected in Sant Cugat (Aranda *et al.* 2006). Originally from Southeast Asia, the entry of invasive mosquito species was already predicted by specialists, due to its uncontrolled spread across continents and because the country holds the environmental conditions for a potential establishment of the species (Eritja *et al*. 2005). During previous years, since 2002, the local Health Care Centre of Sant Cugat has observed a dramatic increase of medical consultations because of insect bites (Giménez *et al*. 2007).

Given the evidence that there was a problem caused by a pest of insects, the City Council of the municipality ordered a diagnosis to the Mosquito Control Service of Baix Llobregat. Thus, on August 11th, 2004, a first report mentioned the discovery of a specimen of the genus *Aedes* mosquito, and it was confirmed in September of the same year that the mosquito was the invasive *Aedes albopictus*. In the autumn of 2004, it was confirmed that the species was also established in the town of Cerdanyola.

During 2003-2004, forty-five points for reception of imported used tires distributed in 25 provinces were monitored through the collection of larvae (Roiz *et al.* 2007-a). The study was conducted in the areas most vulnerable to the arrival of non-native mosquito species, where the tires came from countries already infested. However, against the expectations, this study did not detect any evidence of the establishment of *Aedes albopictus* in the peninsula through the importation of used tires product.

Due to the existence of two international highways in the area of introduction the hypothesis of a dispersal of species through vehicles has been suggested, presumably coming from Italy, but the origin of the Spanish population of the species is not confirmed (ECDC 2009; Roiz *et al.* 2007-a). Genetic analysis (mitochondrial DNA isoenzimes and microsatellites) confirmed a Non-Asian origin, therefore, this species came from America or Europe (Roiz, non-published data).

The detection of the invasive species in Sant Cugat motivated the creation of a working group composed by Public Health and Environment officials of the City Council, from the Province (Diputació de Barcelona) and from the regional government (Generalitat de Catalunya). Also the technical personnel from the Mosquito Control Service of Baix Llobregat and a researcher from Institut Carlos III of Madrid participated in the meetings and through the EVITAR Network (Infectious Diseases Virosis Transmitted by Arthropods and Rodents) a study was conducted (September-November 2004) to determine the distribution and estimate the relative abundance of the species in the municipality. In addition, the identification of the possible expansion into neighbouring municipalities was examined. (Roiz *et al.* 2007-b).

Since 2005, the Regional Government (Generalitat de Catalunya) has carried out a program of vector surveillance, by monitoring traps in the affected and its adjacent municipalities, aiming to find out information about the distribution and the temporal evolution of the species in the territory. The annual results are published in reports that can be assessed on the government website.

Source reduction programs have been carried out in the municipality of Sant Cugat del Vallès since 2006, combined with other information measures through the distribution of printed material in schools and in some public premises. In the following the steps for the decision to implement an integrated vector management control in the area will be detailed.

4.2. Decision process

Since 2006, with the support of a grant from the regional Government to contract civil agents the city council has promoted "door-to-door" source reduction campaigns (SR): civil agents went house-to-house to give support to householders, educate them about ways to avoiding larval breeding in domestic containers.

Despite the benefits achieved by the "door-to-door" source reduction campaigns, numerous citizens' complaints were still reaching the city council every summer. Societal concern was growing, many publications about the loss of quality of life due to the tiger mosquito nuisance appeared in citizen blogs in the local press.

Also, during 2006-2007 it was found that several road scuppers were infested with mosquito larvae, often of this invasive species. Then it was concluded that despite the fact that source reduction is a basic action for mosquito control, other interventions should be implemented to decrease the density of mosquitoes in the area. Moreover, it was necessary to monitor the improvements achieved in the different neighbourhoods of Sant Cugat through trapping systems.

Thus, an agreement was established between the Environmental Science and Technology Institut (ICTA) of the Universitat Autònoma de Barcelona and the City Council of Sant Cugat de Vallès, with the support of the Research Foundation Mútua Terrassa (Universitat de Barcelona, Barcelona) and the Wetland Ecology Department (Doñana Biological Station) and a study was designed to assess the effectiveness of combined interventions implemented in this affected municipality.

The research group in which the author was in charge of municipal technician decided to design a strategy based on Integrated Vector Management (IVM) due to its demonstrated sustainable methodology for long term achievements (Erlanger *et al*. 2008).

Thus, during 2008-2009 four combined interventions have been implemented in some areas of Sant Cugat, the effectiveness of the strategy was assessed by the installation of ovitraps in treated and untreated (control) areas. To complement the interventions performed in the municipality, in 2010 larval surveys were included in the program, being carried out during the door-to-door campaign. The objective was to find out the tiger mosquito's preferred containers and to calculate traditional larval indices, Breteau index (BI), container index (CI), and house index (HI), which have been used as epidemiologic indicators of dengue transmission in endemic regions (Erlanger *et al.* 2008), and larval density with Strickman and Kittayapong methodology (2002).

The outcome of those 3 studied years allowed the design of an annual protocol for the municipality of Sant Cugat del Vallès. The standard interventions aiming at decreasing mosquito nuisance in the area are planned through the year to best match the activity cycle of the insect, with the objective of. Collaboration of citizens is an essential factor to the feasibility and success of protocol implementation.

4.3. Methods

4.3.1. Study site

The study was performed in Sant Cugat del Vallès (41◦28’4’’N, 1◦53’49’’E, 48.32km^2; mean elevation 172m), population 81,901 inhabitants and 30,000 dwellings (Information obtained at City Hall 2010). Sant Cugat has many parks and large areas of single houses with private gardens, courtyards and pools. The average annual rainfall is 605 mm, and the average minimum temperature is 10.2 ºC, with a typical Mediterranean climate. Sant Cugat is located at 15 km northwest of Barcelona, from which the urban area are biogeographically separated by the Natural Park of Collserola. Its geographic situation is depicted on Figure 19.

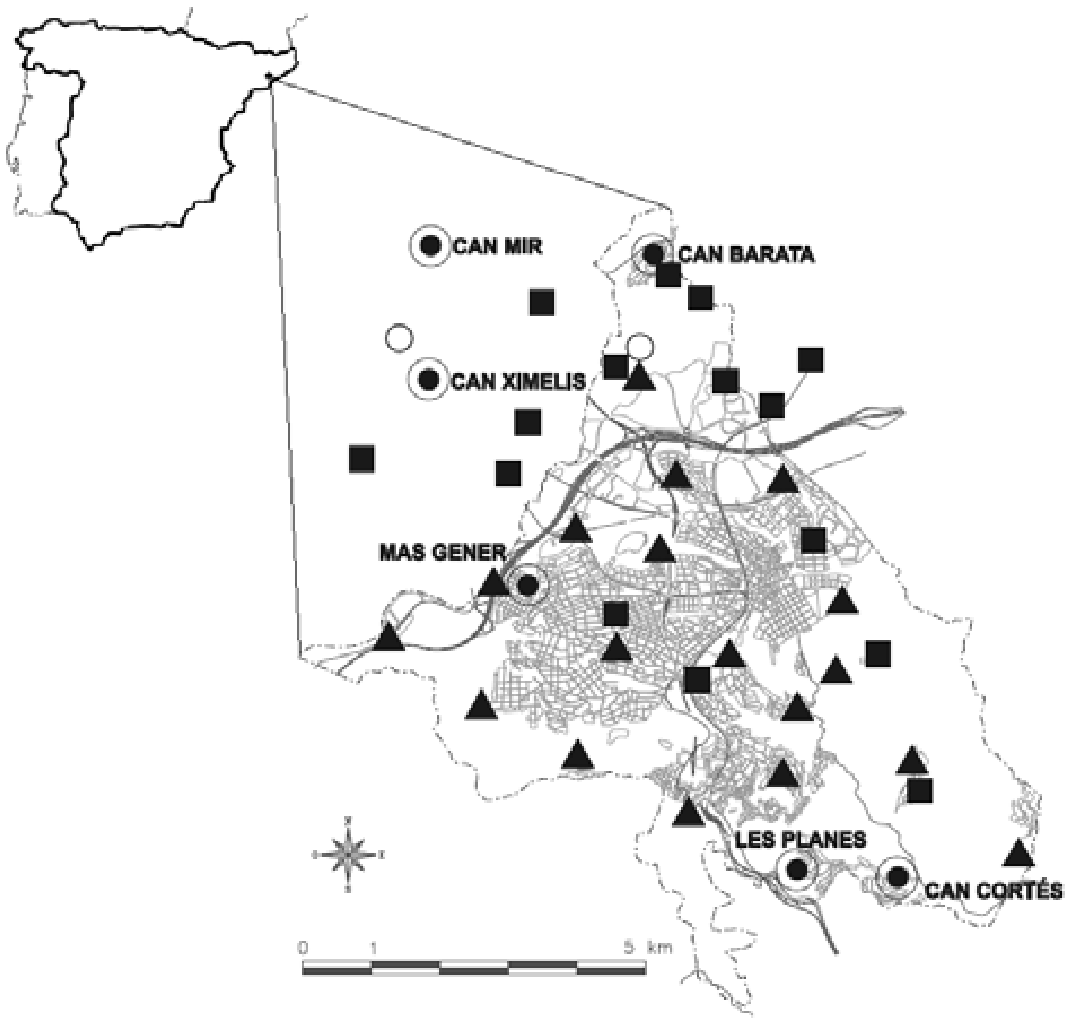
Table 6 - Characteristics of study areas under the combined interventions strategy performed during 2008 and 2009.

	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6	Other areas*
Name of area	Les Planes	Can Cortès	Mas Gener	Can Barata	Can Ximelis	Can Mir	Sant Cugat
Area (x10^3 m^2)	80.5	73.3	60.2	67.7	60.0	60.2	48.32
Number of dwellings	334	100	470	232	150	280	30.000
Treatment 2008	Intervention	Intervention	Control	Control	-	-	Standard
Treatment 2009	Intervention	Intervention	Reintervention	Reintervention	Control	Control	Standard

*Standard area - Total area of Sant Cugat del Vallès

During the implementations combined interventions strategy was carried out in 2008-2009, 4 subareas of Sant Cugat (Les Planes, Can Cortès, Mas Gener and Can Barata) and 2 (Can Ximelis and Can Mir) of the adjacent municipality of Rubi (41◦29’36’’N, 02◦01’57’’E, 32.30km^2; mean elevation 123 m) were studied. The studied zones are depicted in Figure 19 and Table 6.

Figure 19 - Geographic situation of the zones under study.



Geographic situation of the zones under study (see Table 1 for more details). The black triangles indicate the locations of oviposition traps in the standard area of the municipality of Sant Cugat del Vallès in 2008. The black squares and white circles represents respectively the locations of oviposition traps (2006-2010) and BG-Sentinel traps monitored by the Generalitat de Catalunya.

Areas 1 and 2 had intervention for two consecutive years: 2008-2009. During 2008, areas 3 and 4 were used as controls, but after the city council carried out

Source Reduction (SR) program in all houses of Sant Cugat where citizens had complained or asked for technical support, they were included in 2009 as intervention areas. Control zones (areas 5 and 6) were located in Rubí, where the tiger mosquito was detected later and the city council had not promoted any *Aedes spp*. control programs at the time of the study.

The study areas comprised mainly single-family dwellings. They are separed from surrounding neighbourhoods by large roads, woodlands or building complexes. There are between 100 and 470 houses in the selected neighbourhood, and the mean lot size is 0.17–0.25 ha. All housing and inhabitants in the six study areas were included, and only people who refused to participate in the study, those with mental disabilities and those <16 years were excluded.

4.3.2. Combined Interventions Strategy

This was a quasi-experimental study with multiple interventions, performed from February to October in 2008 and from May to December in 2009, and consisted of four complementary strategies. The first of these was Source Reduction (SR). House-to-house visits were carried out in each studied neighbourhood. The field workers asked for permission to enter the properties to inform and educate the citizens about measures to reduce mosquito abundance and prevent mosquito-borne disease.

SR achieved through environmental sanitation of containers was used as a method for experimentally manipulating the production of immature *Ae. albopictus* in container habitats. As many residences as possible within neighbourhoods were surveyed for water-holding containers. In intervention areas, any water remaining in a container was discarded, and the container was turned over so that it would not collect rainwater. Any wet containers that could not be emptied were treated with an insect growth regulator larvicide (diflubenzuron 2% at a concentration of 1 g/hl) (Flower, Lleida, Spain). SR measures were conducted after adult householders gave verbal informed consent. The outcome of each visit was recorded in detail on a form (See annex 2) and given to the city council.

The second measure was larvicide treatment with DEVICE TB2 (diflubenzuron 2% at 1 g/hl) in scuppers, water tanks and street drains containing stagnant water in the intervention areas. A granular formulation of the biolarvicide *Bacillus thuringiensis israelensis* (Bti), Vectobac G (EPA Registration No. 73049-10) (1.2% Bti, 1 g/m2) (Valent Bio-Sciences Corporation, Libertyville, IL, USA), was applied to seasonal streams.

The third measure was sanitization of municipal sites and wooded terrains, with removal of uncontrolled rubbish dumps in the intervention zones. The fourth measure was adulticide treatment (Fastac 10% – alfacipermetrin 50 cc/hl) (Basf Española S.A., Tarragona, Spain). In September 2008 and monthly, from July to October 2009, insecticide was sprayed on the vegetation of some public gardens of each neighbourhood of the study area by specialized teams. These isolated treatments were carried out, selecting two or three points in each intervention area, and giving priority to public gardens with the greatest number of users, as well as points located centrally in the intervention area to achieve a greater effect (Baseggio 2008).

In 2010 the four combined actions strategy was not applied, but from August to October of this year, educators and inspectors performed house-to-house visits (Source Reduction) in areas of Sant Cugat, carrying out the program for prevention of tiger mosquito presence. In each studied area 3 or 4 sub areas of 40,000 m^2 (200x200 m) were randomly selected (Epidat 3.0) for the inspections.

The team also visited other districts of the municipality (standard area) when residents required technical support from the Local Council. In the three studied years a resident of each inspected household was interviewed, usually the person who was more cooperative, and excluding children under 16 and people without the cognitive ability to participate.

4.3.3. Environmental data and surveys

A household was defined as one separate unit of accommodation (individual home or apartment) and the immediate surrounding outdoor zone (garden or back yard), irrespective of the number of people residing within the unit. Information about housing type (principal domicile, secondary residence/empty house or service) from each household of the studied zones was collected. Also, some of the environmental characteristics of the households were observed and data was collected on the inspection sheets (See annex 2).

Environmental characteristics observed included: presence of garden, courtyard, pool, vegetable garden, coop, terrace, building works as well as the presence of abundant vegetation, stacked materials, solid waste, stacked pruning or firewood. All those factors were documented during the inspections as they may influence the abundance of mosquitoes in the household. All those factors were documented during the inspections as they may influence the abundance of mosquitoes in the household.

Data about potential and active foci was also collected during the inspections. Potential foci was defined as open containers that accumulated or could accumulate water but where larval growth could not be detected in the moment of the inspection. Active foci were the larval breeding containers were larval growth was detected, also referred to as larval habitats.

Environmental data from technical observation were collected from inside each visited property, and if not, by observation from outside. Households inspected from within or with good visibility from outside were classified according to their risk level of exposure to mosquito nuisance (to mosquito breeding and/or shelter). It was categorized that a house had a high risk of exposure when there were detected spots with stagnating water (potential focus or potential larval breeding) and overdeveloped grass or shrub vegetation, medium risk when there were points that could collect water but the garden was well maintained, and low risk when there was no spot that could accumulate water. Finally, there was no risk when there was no outdoor site in the housing and no spot with the possibility of an accumulation of water. Data was also collected from surrounding areas of each property (200 m around the external limits).

Moreover, in 2010 occurrences of adult tiger mosquitoes detected during the visits were recorded. Data collected during inspections and interviews were filed at the City Hall.

Neighbours from the whole municipality of Sant Cugat could also ask for technical support from the city council. All cases reported in this municipality were attended to with standard support: a civil agent inspected the household and SR measures were applied at the affected house and in all the houses that surrounded it; householders were interviewed and data were collected. The area and procedure are referred to in our results as "standard".

Good accessibility was defined as the situation in which the surfaces of the back yard or garden of the household were mostly visible even if the field workers could not enter it. When there was nobody at home at the time of the visit or when the citizens would not collaborate with the civil workers at the time of the inspection, an official letter emphasizing the need for collaboration with written and illustrated advice was sent, explaining how to avoid mosquito reproduction in the dwellings.

Educational printed materials with illustrated recommendations were used and distributed to every visited household. In the control areas (not intervened), data were collected from some randomly selected houses, and in those cases the inspections were carried out from outside the dwelling at the end of the season.

4.3.4. Monitoring methods

A. Ovitraps

About 15 oviposition traps (ovitraps) were positioned in each of the six study areas, approximately 200 m one from the other, in a total of 94 ovitraps. Each sample station consisted of one ovitrap, a black plastic cup with a diameter of 14cm filled with 300 ml of clean water and containing a half-immersed piece of wood measuring 2.5×12.5 cm. The ovitraps were placed in sites shaded by vegetation in both treated and untreated neighbourhoods.

The habitat characteristics of the survey station are fundamental to the effectiveness of the ovitraps, previous studies demonstrate that traps set at ground level, protected from wind and direct sun and also by vegetation are more suitable (ECDC – Guidelines for the surveillance of invasive mosquitoes in Europe –2012, Craig *et al.* 2006; Russell and Ritchie 2004). The paddles were collected fortnightly and placed in individual plastic drawers that were sealed with parafilm and labelled. The water in the ovitrap was always checked for hatched mosquito larvae and/or pupae. Fresh tap water, new paddles and missing ovitraps were systematically replaced. Biolarvicide *Bti* was applied to prevent the production of mosquitoes in the trap. The number of eggs collected per trap was assessed by examination under a stereomicroscope (40×). All the surfaces, including the edges, were checked and the eggs were counted.

Population abundance was expressed by the mean and median number of eggs per positive trap. Because a small portion of the eggs laid (less than 5%) could belong to other tree hole *Aedes* mosquitoes (*Ae. geniculatus*, *Ae. echinus* or *Ae. berlandi*) (Zamburlini and Frilli 2003) we raised them in the laboratory based on Roiz *et al.* (2008) to obtain larvae to confirm the identification of individuals. All the emerged larvae were identified as *Ae. albopictus*. Those results, together with other studies in the same area (Roiz *et al.* 2008) confirmed that the eggs in the ovitraps were *Ae. albopictus*.

Ovitrap monitoring was performed from August to October in 2008 and from May to December in 2009. The months with greatest activity of *Ae. albopictus* were always studied, but with variation in the follow-up procedures, due to changes in the resources available each year. In 2008, as well as in the study zones, 18 ovitraps were homogeneously distributed through the town, corresponding to the standard municipality area. In 2009, the standard area was not monitored by ovitraps.

B. Larval Surveys and larval Index

Larval surveys were carried out from August to October 2010 and were conducted by examining all containers in every house in the study areas. Potential larval habitat (standing water container) and active mosquito breeding containers with growth of pupae and larvae were identified and assessed. Small receptacles containing larvae were emptied directly into sample jars (1 l). When the container volume was greater than 1 l, the collection was done through the filling a dipper 5 times (200 ml). The filling was homogeneous, positioning the dipper on the surface of the container and moving slowly without actually sinking it, sampling all the edge of the surface area of the container. The samples were brought to the laboratory for identification and counting.

Larval Index: Abundance of larvae in a container was recorded in four categories: absent, low (1-9 larvae), moderate (10-50 larvae) or high density (>50 larvae), based on Strickman and Kittayapong (2002). The larval index was calculated as the sum of larvae in all containers of a single house, and was an estimate of number of *Aedes* spp. larvae per dwelling. Traditional indices such as Breteau index (BI), container index (CI), and house index (HI) were also calculated. The BI is defined as the number of positive containers per 100 houses, the HI is defined as the number of positive houses per 100 houses and the CI is defined as the number of containers with immature stages per 100 containers with water (Erlanger *et al*. 2008). The larval index calculations were made considering only the data of inspections where access to the property was possible.

4.3.5. Statistical Analysis

In the following the tests used in the statistical analysis is detailed:

Results for the qualitative variables observed during the inspections in the dwellings and interviews were expressed as absolute values and percentages. The values for quantitative variables were expressed with at least one measurement of central tendency (mean and median) and one of dispersion (standard deviation and range). The normality was explored with the Kolmogorov-Smirnov test.

In bivariate analysis, variables were compared using the χ^2 test, and the quantitative ones with the t-test for parametric ones and Mann–Whitney U test for non-parametric. For significant variables, 95% confidence intervals (CI) were established.

The level of statistical significance was set to 0.05.

For the construction of multivariate model environmental characteristics, the dependent variable was active foci (in a premise) taking into account its categories "Yes" or "No". The category "No information" was considered as a missing value. Independent variables were selected according to their significance in the bivariate statistical analysis. The ones with high biological plausibility were included. The model regression logistic was used with method enter, as this method may be the more simple one that allows adjustment for all the variables introduced in the model.

Data on the number of eggs counted in the ovitraps were analysed with a negative binomial distribution generalized linear model (GLM). Negative binomial and Poisson are correct methods for counting data. In this case, a negative binomial was used rather than Poisson distributed error to reduce model overdispersion caused by the aggregation of captures. Missing data were replaced using the method of the median of adjacent points. The response variable was egg abundance, the intervention type is an explanatory variable (factor) and month and area were introduced as covariates to control for their possible effect and to centre the analysis on the effect of the different level of the treatments.

Statistical analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA).

4.3.6. Ethical considerations

Verbal informed consent of the citizens was obtained. The present study was done in accordance with the guidelines of Research Ethics Committee of Mútua Terrassa Hospital.

4.4. Results

4.4.1. House-to-house visits - Source reduction campaign.

During the years 2008-2010, 3720 households were inspected, 69% were first residence, 12% inhabited or second residence, and the rest mainly installations and facilities, such as schools (n=48) and sport centers (n=9), stores and also sites without buildings. The studied areas had a large number of households with gardens (61%) and courtyards (62%), with an average number of 2 outdoor zones per property (mean number of outdoor areas per household: 2±1 [0-7]), which were carefully inspected by field workers. The main environmental characteristics favouring the abundance of adult mosquitoes which were recorded for households and their surroundings are depicted in Table 7 and Table 8.

The accessibility to gardens and other outdoor zones varied between 13% and 29% of dwellings, according to the studied year. Of the total number, 691 were inspected from the inside, and another 1306 satisfactorily from the outside, totalling 1997 households with adequate visibility of the outdoor zones.

Over a total of 3720 households, high, medium and low risk of exposure for mosquitoes within the properties were 791 (21%), 763 (21%) and 787 (21%), respectively, with only 45 (1%) with absent risk and 1334 (36%) in which no category could be assigned for lack of verified data, without significant variation over the 3 years. In 2010, detections of specimens of tiger mosquito in aerial phase were documented in 27% of the inspected premises.

Due to the intrinsic characteristics of the premises, water was permanently stored in open tanks at 1% of households (n=45); the breeding spots had to be neutralized periodically, usually with larvicides. In the three study years at least one breeding point was detected in the environment surrounding the visited households.

For potential and active larval points of production and development found at premises, results are shown in Table 9.

In the three study years, it was detected that 266 households (7% of the total inspected) had at least one container infested with mosquito larvae. In 205 dwellings, those infested receptacles could be neutralized at the time of the inspection.

Larval growth was especially detected in premises that had scuppers, drums (usually with volume of about 200 l) and old pools with stagnant rain water without chlorine (used mainly for the watering the garden).

Table 7 - Characteristics of the inspections, housing type and risk level of exposure. Environmental data from door-to-door survey.

Section I - Characteristics of the inspections and households and other buildings and sites visited				
Year (n=Total of households)	2008 (n=2104)	2009 (n=1000)	2010 (n=616)	Total (n= 3720)
1. Inspections	n (%)[a]	n (%)[b]	n (%)[c]	n (%)[d]
- From the inside the household	274 (13%)	289 (29%)	128 (21%)	691 (19%)
- From the outside	1830 (87%)	711 (71%)	488 (79%)	3029 (81%)
- Total surveys with good visibility of the households backyards	1157 (55%)	586 (59%)	254 (41%)	1997 (54%)
2. Housing types surveyed				
- First home Site (without building)	1573 (75%)	589 (59%)	369 (60%)	2531 (69%)
- Empty house (second family home, uninhabited or house in ruins)	75 (4%)	76 (7%)	26 (4%)	177 (5%)
- Site (without building)	105 (5%)	34 (3%)	35 (6%)	174 (5%)
- Service Building (Schools, stores and others)	49 (2%)	34 (3%)	35 (6%)	118 (3%)
3. Environmental characteristics of outdoor housing area				
- Courtyard	1113 (53%)	782 (78%)	393 (64%)	2288 (62%)
- Garden	1185 (56%)	719 (72%)	361 (59%)	2265 (61%)
- Pool treated with chlorine	185 (9%)	221 (22%)	100 (16%)	506 (14%)
- Woodland	161 (8%)	64 (6%)	27 (4%)	252 (7%)
- Vegetables garden	127 (6%)	134 (13%)	78 (13%)	339 (9%)
- Terrace	30 (1%)	217 (22%)	61 (10%)	308 (8%)
- Community Zone	29 (1%)	40 (4%)	17 (3%)	86 (2%)
- Building site	23 (1%)	9 (1%)	18 (3%)	50 (1%)
- Coop	22 (1%)	14 (1%)	11 (2%)	47 (1%)
Section II: Exposing Risk level of housing (excluding public roads and external environment)				
- High	372 (18%)	376 (38%)	143 (23%)	791 (21%)
- Medium	547 (26%)	141 (14%)	75 (12%)	763 (21%)
- Low	520 (25%)	186 (19%)	81 (13%)	787 (21%)
- None	15 (1%)	24 (2%)	6 (1%)	45 (1%)
- Not possible to specify	650 (31%)	373 (38%)	311 (50%)	1334 (36%)

[a] Per cent per total number of visits in year 2008
[b] Per cent per total number of visits in year 2009
[c] Per cent per total number of visits in year 2010
[d] Per cent per total number of visits in the three study years 2008-2010

Table 8 - Resting sites and surrounding characteristics - Environmental data from door-to-door survey.

Factors that may increase the abundance of mosquitoes at aerial phase in the household (resting sites of aerial stage)				
Year (n=Total of households)	2008 (n=2104)	2009 (n=1000)	2010 (n=616)	Total (n= 3720)
1. Resting sites of the outdoor areas in the households	n (%)[a]	n (%)[b]	n (%)[c]	n (%)[d]
- Abundant Vegetation	734 (35)	164 (16)	94 (15)	992 (27)
- Materials stacked and waste	209 (10)	116 (12)	99 (16)	424 (11)
- Stacked pruning or firewood	261 (12)	110 (11)	102 (17)	473 (13)
2. Environmental factors of the area (200 m external surrounding household)				
- Stream	326 (15)	994 (99)	552 (90)	1872 (50)
- Abundant vegetation	982 (47)	555 (56)	614 (100)	2151 (58)
- Uninhabited house	163 (8)	221 (22)	145 (24)	529 (14)
- Uncontrolled landfills	45 (2)	6 (1)	64 (10)	105 (3)
- House with vegetable garden or house with work.	179 (2)	85 (9)	49 (8)	313 (8)
- Spots with stagnant water. Number (%)	909 (43)	539 (54)	547 (89)	1195 (32)
- Spots with stagnant water Median (range)	3 (1 – 24)	3 (1 – 15)	2 (1 - 24)	3 (1 – 24)
- Spots of larval growth. Number (%)	106 (5)	31 (3)	31 (5)	168 (5)
- Spots with larval growth Median (range)	3 (3 – 15)	3 (1 – 15)	1 (1-15)	3 (1 - 15)
- Premises with active focus periodically neutralized.	28 (1)	8 (1)	9 (1)	45 (1)

[a] Per cent per total number of visits in year 2008
[b] Per cent per total number of visits in year 2009
[c] Per cent per total number of visits in year 2010
[d] Per cent per total number of visits in the three study years 2008-2010

Although other smaller containers like flowerpot saucers and other objects (e.g.: Toys, ashtrays, and other) were frequently found in the gardens, larval growth was not frequently detected in those spots, possibly because they dried before completing the biological cycle of the insect.

The great majority of visits had been carried out in first family residences, where families lived all the year. The majority of larval habitats had been found in these premises (50%), followed by empty houses (including the second second family home, uninhabited or house in ruins) (14%), and commercial premises (13%).

Table 9 – Potential and active larval habitats detected in households during the 2008-2010 surveys.

	Potential larval mosquito habitats				Active larval mosquito habitats			
Year	**2008**	**2009**	**2010**	**TOTAL**	**2008**	**2009**	**2010**	**TOTAL**
Total of households	**(n=2104)**	**(n=1000)**	**(n=616)**	**(n=3720)**	**(n=2104)**	**(n=1000)**	**(n=616)**	**(n=3720)**
Positive n (%)	920 (44)	530 (53)	302 (49)	1752 (47)	58 (2)	123 (12)	85 (14)	266 (7)
Positive containers per household Median (range)	3 (2-8)	3 (1-6)	3 (1-20)	3 (1-20)	3 (1-4)	2 (1-3)	1 (1-3)	1 (1-4)
Negative n (%)	506 (24)	191 (19)	54 (9)	751 (20)	897 (42)	322 (32)	108 (18)	1327 (36)
No information n (%)	678 (32)	279 (28)	260 (42)	1217 (33)	1149 (55)	555 (56)	423 (69)	2127 (57)
Type of recipient n (%)								
Drums	163 (8)	100 (10)	89 (14)	352 (9)	20 (1)	28 (3)	20 (3)	68 (2)
Scuppers (drains)	142 (7)	119 (12)	60 (9)	321 (8)	20 (1)	31 (3)	23 (4)	74 (2)
Pools (not treated)	124 (6)	107 (11)	84 (13)	315 (8)	10 (1)	40 (4)	21 (3)	71 (2)
Solid Waste	188 (9)	132 (13)	74 (1)	395 (11)	9 (<1)	14 (1)	11 (2)	34 (1)
Small objects [a]	156 (7)	123 (12)	104 (17)	386 (10)	7 (<1)	9 (1)	10 (2)	26 (1)
Flowerpot saucers	332 (16)	257 (26)	68 (11)	657 (18)	6 (<1)	15 (2)	5 (1)	26 (1)
Ornamental fountains	76 (4)	71 (7)	36 (5)	183 (5)	4 (<1)	12 (1)	4 (1)	20 (1)
Other receptacles[b]	27 (1)	58 (6)	30 (4)	115 (3)	4 (<1)	6 (1)	12 (2)	22 (1)

Per cent of number of premises with potential or larval foci per total of visited premises each study year (2008-2010) including totals

[a] Toys, ashtrays, and other; [b] Boat, furniture, ornamental elements

Larval habitats and adult mosquitoes (aerial phase) were found more frequently in premises with some specific environmental characteristics; the main results are depicted in Table 10.

Within premises with larval habitats (active foci), comparing to the ones free of larval breeding, the following factors were detected more frequently: 15% more premises with vegetable gardens (CI 95%: 9-20%); 15% with stacked material (of building or gardening) in the garden (CI 95%: 9-20%); 13% more possessing solid waste (CI 95 %: 9-17%); 10% more with scuppers in the garden (CI 95 %: 5-16%); 6% more sites with firewood stacked in the garden (CI 95%: 2-10%); 5% more with coops (CI 95%: 2-8%) and 5% more with building works in the garden (CI 95%: 2-7%).

Among premises with detected adult mosquito, comparing to the ones free of them, it was detected more frequently the following characteristics: 15% more with vegetable gardens (CI 95%: 9-20%); 10% more premises with solid waste (CI 95%: 3-15%); 9% more premises with scuppers (CI 95%: 3-15%); 9% more premises with stacked materials (CI 95%: 3-15%); 8% more premises with vegetable garden (CI 95%: 2-15%), 8% more premises with (uncontrolled) abundant vegetation (CI 95 %: 1-15%) and 3% more with coops (CI 95%: 2-6%).

Moreover, larval habitats were detected more frequently in some types of premises, as well as adult mosquitoes. Within premises with larval habitats (active foci) comparing to the ones free of larval breeding, it was detected more frequently in the following type of premises: there are 11% more of commercial premises (mainly garden stores) (CI 95%: 7-15%), 10% more educative centers (CI 95%: 6-14%) and 3% site with work (new building) (IC 95%: 1-6%).

With regard to the detection of adult mosquitoes, they were observed in 6% more schools (CI 95%: 2-11%) and 6% more commercial premises (mainly garden stores) (CI 95%: 2-11%).

Pure woodlands have not been associated with the detection of breeding spots or with the presence of adults.

Table 10 – Environmental characteristics of premises as risk factors associated with vector production (presence/absence of larvae/adults).

	Door to door campaign 2008-2010 (n=3720)			Door to door campaign only 2010 (n=616)	
Risk factor	Premises with larval habitats n=266	Premises without larval habitats n=1327	Premises (unknown)* n=2127	Premises with adult mosquitoes n=165	Premises without adult mosquitoes n=451
Section I – Environmental characteristics of premises as risk factor for mosquito production					
Vegetable garden[a,b]	65 (24%)[c]	132 (10%)[d]	142 (7%)[e]	31 (19%)[f]	47 (10%)[g]
Stacked material[a]	59 (22%)[c]	101 (8%)[d]	184 (9%)[e]	27 (16%)[f]	46 (10%)[g]
Solid Waste[a,b]	41 (15%)[c]	33 (2%)[d]	76 (4%)[e]	27 (16%)[f]	29 (6%)[g]
Scuppers [a,b]	75 (28%)[c]	236 (18%)[d]	346 (16%)[e]	26 (16%)[f]	30 (7%) [g]
Stacked firewood[a,b]	31 (12%)[c]	74 (6%)[d]	87 (4%)[e]	24 (15%)[f]	23 (5%)[g]
Coop[a,b]	16 (6%)[c]	16 (1%)[d]	15 (1%)[e]	7 (4%)[f]	4 (1%)[g]
House with work[a]	15 (6%)[c]	15 (1%)[d]	20 (1%)[e]	3 (2%)[f]	15 (3%)[g]
Abundant Vegetation[b]	99 (37%)[c]	419 (32%)[d]	474 (22%)[e]	35 (21%)[f]	59 (11%)[g]
Sección II – Premise type as risk factor of mosquito production					
First home	134 (50%)[c]	990 (74%)[d]	1499 (70%)[e]	107 (64%)[f]	271 (60%)[g]
Empty house	36 (14%)[c]	127 (10%)[d]	237 (11%)[e]	16 (10%)[f]	46 (10%)[g]
Commercial premises[a,b]	32 (13%)[c]	18 (<1%)[d]	18 (<1%)[e]	13 (9%)[f]	7 (2%)[g]
Educational centers[a]	31 (12%)[c]	17 (<1%)[d]	0	7 (4%)[f]	8 (2%)[g]
Site	18 (7%)[c]	83 (6%)[d]	35 (2%)[e]	7 (4%)[f]	22 (5%)[g]
Site with work (New build site)[a]	12 (5%)[c]	17 (<1%)[d]	29 (2%)[e]	7 (4%)[f]	5 (1%)[g]
Sports Centers	4 (2%)[c]	4 (<1%)[d]	1 (<1%)[e]	2 (1%)[f]	0
Woodland	2 (1%)[c]	24 (2%)[d]	20 (1%)[e]	4 (2%)[f]	23 (5%)[g]

* No information was obtained about the presence/absence of mosquito breeding container (active focus); [a] Statistical differences in relation to the presence of larval active foci. ($p<0.05$); [b] Statistical differences in relation to the presence of adult mosquitoes. ($p<0.05$); [c] Per cent of number of premises with risk factor and larval foci per total of premises with larval foci; [d] Per cent of number of premises with risk factor without a larval foci for total of premises without larval foci; [e] Per cent of number of premises with the risk factor with no information about larval foci for total of premises with no information about larval foci; [f] Per cent of number of premises with the risk factor and presence of adult mosquito for total of premises with presence of adult mosquito; [g] Per cent of number of premises with risk factor and absence of adult mosquito for total of premises with absence of adult mosquito.

The significative environmental variables in relation to active larval foci obtained in the multivariate analysis are depicted in Table 11.

Table 11 - The significative environmental variables obtained in the multivariate analysis.

Variable	B	Standard deviation	Wald	dF	Sig.	Odds Ratio (OR)	95% C.I.	
							Lower	Upper
Vegetable garden	1.0	0.2	30.1	1	<0.001	2.7	1.9	3.9
Stacked (gardening or building) material	1.0	0.2	26.2	1	<0.001	2.7	1.9	4.0
Coop	1.0	0.4	7.3	1	0.007	2.8	1.3	6.1
Commercial premises (mainly garden stores)	1.0	0.3	9.1	1	0.003	2.6	1.4	5.0
Site with work (new building site)	1.1	0.4	7.9	1	0.005	3.1	1.4	7.0

In the three study years it was observed an association between premises with vegetable garden, stacked materials, coops as well as commercial premises and sites with works (new buildings) with the detection of larval breeding containers.

Finally, in 2010 an association was observed between the premises that had larval foci and the presence of adult mosquitoes. It was found that 66% of households surveyed that had some breeding spot also had some flying mosquitoes. Among these houses with a larval growth container, there were 34% more with mosquitos in aerial phase than without them ($p<0.05$).

4.4.2. The combined complementary interventions 2008-2009

Results showed a significant reduction in numbers of mosquito eggs in treated areas compared with untreated areas in 2008 and 2009 (Figure 20 and 21). However, the tiger mosquito continues to expand its range, in 2008, the accumulate medians of eggs were 175 and 272 in the intervention and control areas, respectively. In 2009, these medians were 884 and 1668 eggs.

Figure 20 - Evolution of the medians of eggs of *Aedes albopictus* with fortnightly sampling from August to October 2008.

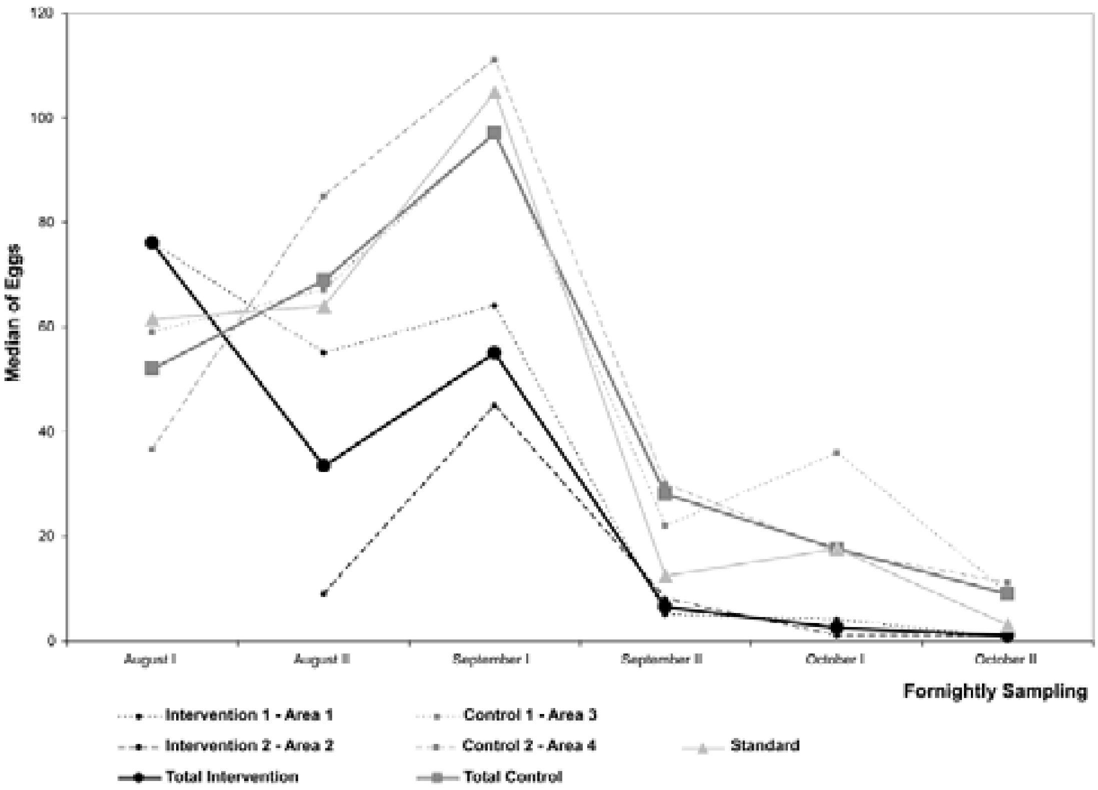
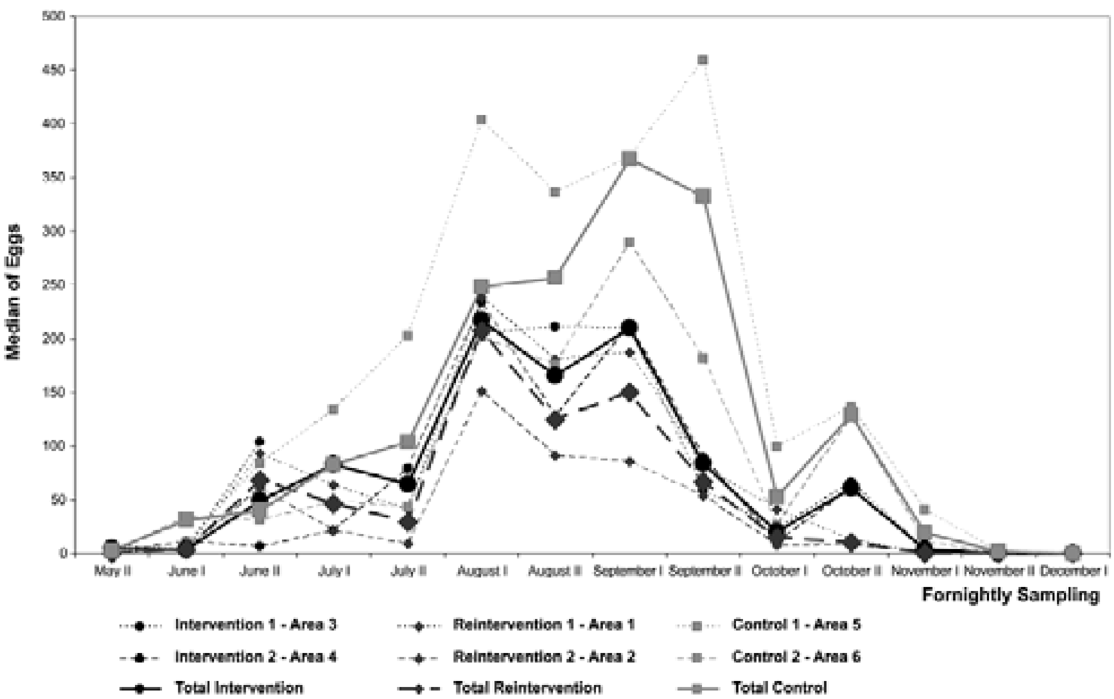


Figure 21 - Evolution of the medians of eggs of *Aedes albopictus* with fortnightly sampling from May to December 2009.



The resulting model after statistical analysis is shown in Table 12. All three studied variables (intervention, area and month) affected the egg abundance ($P < 0.05$). Control areas showed significantly higher egg abundance than the other areas. Intervention areas also presented significantly higher egg abundance than the reintervention areas.

Table 12 - Results of the fortnightly sampling in 2009 using the negative binomial distribution generalized model analysis.

Response variable			*β (IC95%)*	*Std. Error*	*Wald Chi-Square*	*df*	*Sig.*
Egg abundance	Intercept		2.099 (1.643-2.555)	0.2324	81.54	1	<0.001
	Factor	Control	0.721 (0.572-0.869)	0.0757	90.569	1	<0.001
		Intervention	0.155 (0.004-0.307)	0.0773	4.025	1	0.045
		Re-intervention[a]					
	Covariates	Area	0.047 (0.011-0.084)	0.0186	6.445	1	0.011
		Month	0.287 (0.233-0.341)	0.0275	109.216	1	<0.001

a – Reference category

The main findings in the study areas of the four complementary interventions are shown in Table 13. In the study areas, regarding the interventions "larvicide treatment" and "clearance and waste removal", these were similar during 2008 and 2009, but adulticide treatment was applied four times in 2009 and only once in 2008.

Selection of the insecticides used was based on data from recent literature (Ballenger-Browwning & Elder 2009; Basseggio 2008; Heintze *et al.* 2007; Zamburlini & Frilli 2003). Biorational larvicides were used in immature control: the larval growth regulators (IGRs), as Diflubenzuron (DFB) and Piriproxyfen and the microbial larvicide Bacillus thuringiensis israelensis (Bti). DFB and Piriproxyfen were used in scuppers and other artificial receptacles and Bti was applied in pools of temporary streams and puddles.

Table 13 – Complementary interventions performed in 2008-2009 in study areas of Sant Cugat del Vallès.

Intervention		2008 Intervention areas		2008 Control areas		2008 Other areas	2009 Intervention areas		2009 Reintervention areas		2009 Control areas		2009 Other areas
		Area 1 Les Planes	Area 2 Can Cortès	Area 3 Mas Gener	Area 4 Can Barata	Standard	Area 3 Mas Gener	Area 4 Can Barata	Area 1 Les Planes	Area 2 Can Cortès	Area 5 Can Ximelis	Area 6 Can Mir	Standard
Source reduction	Visited houses n (% total dwellings)[a]	296 (89)	48 (48)	61 (13)	18 (8)	1681 (7)	345 (89)	109 (46)	80 (24)	46 (46)	17 (11)	20 (7)	383 (2)
	Accessibility n(%)[b]	145 (49)	33 (69)	39 (64)	13 (72)	926 (55)	174 (50)	61 (56)	25 (31)	13 (28)	14 (82)	14 (70)	281 (73)
	Premises internally inspected n(%)[b]	42 (14)	6 (12)	15 (25)	3 (17)	209 (19)	67 (19)	24 (22)	12 (15)	12 (26)	1 (1)	3 (1)	172 (49)
	Premises with larval habitats n(%)[b]	18 (6)	0	2 (3)	1 (6)	32 (2)	17 (5)	2 (2)	8 (10)	6 (13)	0	0	86 (22)
	Premises with water holding containers n(%)[b]	152 (51)	21 (43)	32 (52)	10 (55)	709 (42)	186 (54)	52 (48)	31 (39)	19 (41)	12 (70)	18 (90)	211 (52)
	Interviews n(%)[b]	91 (31)	11 (23)	17 (28)	5 (5)	303 (18)	70 (20)	27 (27)	20 (25)	12 (26)	4 (23)	6 (30)	107 (28)
Waste removal	Area (ha) (%)[c]	0.5 (0.8)	0.1 (0.1)	0.5 (0.8)	0.2 (0)	-	1 (1.17)	0.4 (0.6)	0.3 (0.4)	0.3 (0.4)	-	-	-
Adulticide treatment	Area (ha) (%)[c]	0.7 (0.9)	0.5 (0.7)	-	-	-	1 (1.17)	0.5 (0.7)	0.5 (0.8)	0.5 (0.7)	-	-	-
Larvicide treatment	Number of public sites with larvicide treatments (l)	1 (2)	1 (2)	-	-	-	15 (50)	-	1 (2)	1 (2)	-	-	-

[a] Total of dwellings in the area (see table 6)
[b] Total of visits in the area
[c] Total of study area (ha) (see table 6)

4.4.3. Larval index

Larval surveys in 128 dwellings performed during 2010 showed that 104 (81%) had containers with standing water, and 56 (44%) had some larval growth container. Mosquito breeding containers were sampled (n=74). It was found that 52 (71%) of these receptacles contained *Ae*. *albopictus* larvae, 22 (30%) had the common house mosquito *Culex pipiens* (Diptera: Culicidae) immature and 7 (10%) of the foci were chironomid larvae (Diptera: Chironomidae). Four (5%) of the receptacles contained both *Ae. albopictus* and *Cx. pipiens*. The main results obtained during the larval survey are detailed in Table 14. The number of premises with specific larval breeding containers is depicted in Figure 22, and the larval densities in the different containers inspected are illustrated in Figure 23.

Table 14 – Larval surveys performed from August to October 2010.

1 - Total number of visited houses 2010	616
2 - Households internally inspected	128 (21%)
3 - Households with potential larval production (standing water or opened containers)	104 (17%)
4 - Total of containers inspected	568
5 - Average number of containers with stagnant water per household	4±5 (1-100)
6 - Internally inspected households with larval breeding containers	59 (9%)
7 - Number of premises with the following species detected - Ae. albopictus larvae - Culex pipien larvae - Quironomidae	 39 (6%) 19 (3%) 5 (1%)
8 - Total of containers with detected *Aedes* immature	52
9 - Category of households by Strickman & Kittayapong (internally inspected) : - High density - Medium density - Low density - Negative	 36 (28%) 2 (2%) 1 (1%) 89 (70%)
10 - Larval index by Strickman & Kittayapong method	65±30 (5-153)
11 - Breteau index	41 % (IC 95%: 32-49%)
12 - House index	31% (IC 95%: 23-38%)
13 - Container index	9 % (IC 95%: 7-12%)

Figure 22 - Number of premises with detected larval breeding of *Aedes albopictus* and *Culex pipiens.*

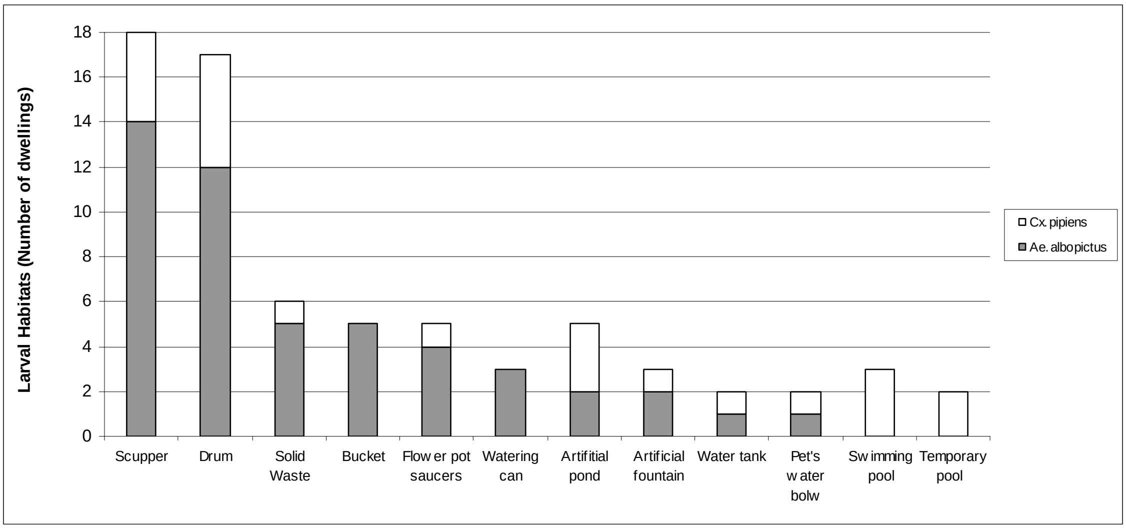
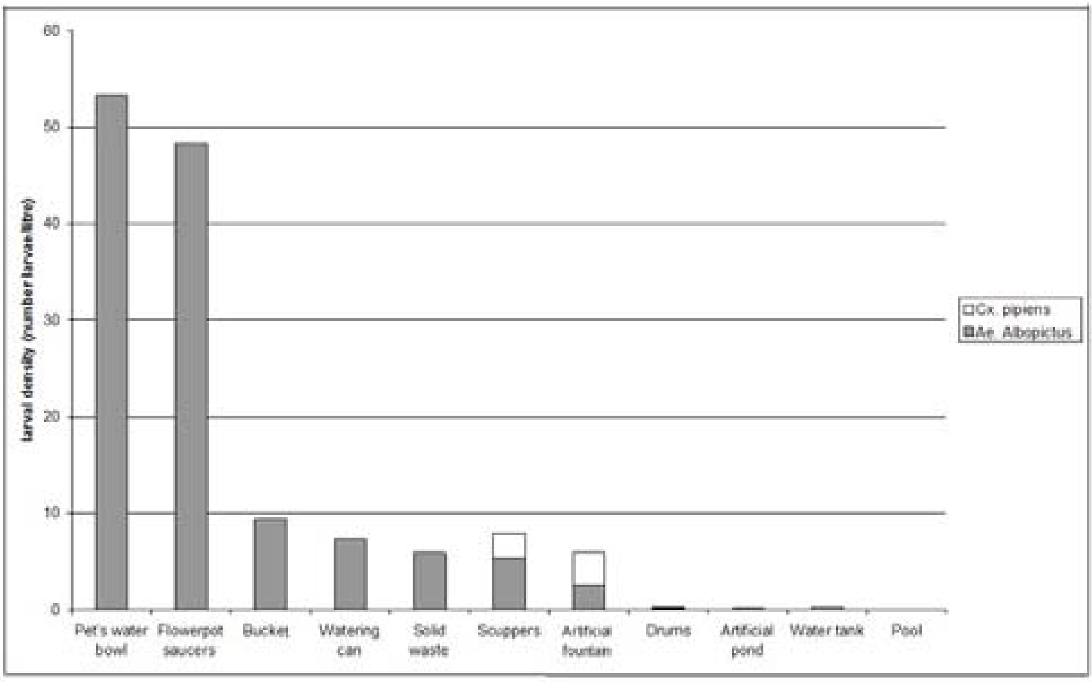


Figure 23 - *Aedes albopictus* and *Culex pipiens*: Larval density detected in the different containers.



During the larval survey, larval habitat containers were found in a higher number of households with scuppers (n=18), followed by dwellings with drums (n=14). Both species, *Aedes albopictus* and *Culex pipiens* were detected in those containers, but the tiger mosquito was found in a greater number of premises. Small recipients were mostly colonized by *Ae. albopictus*, no specimens of this invasive species were detected in pools or inside temporary pools on the ground. In those cases, the common house mosquito larvae were frequently found. Pet's water bowl was the recipient found with highest larval density (54 larvae/l) followed by flowerpot saucers (48 larvae/l).

4.4.4. Questionnaires

During the years 2008- 2010, 820 citizens were interviewed. About 60% were women and the average age was 53±17 years. About 65% had upper-middle schooling and 89% a middle-high socioeconomic level. The socioeconomic characteristics of the population who answered the survey are listed in Table 15. The information collected in relation the perception of people about health effects is presented in Table 16, and the effects on quality of life and knowledge and preventive measures are detailed in Table 17.

Table 15 - Sociodemographic characteristics of the samples studied, detailed by years.

	2008 (n=428)	2009 (n=245)	2010 (n=147)	Total (n=820)
Sex				
- Female	271 (63%)	143 (58%)	81 (55%)	496 (60%)
- Male	152 (37%)	103 (42%)	67 (46%)	326 (40%)
Age	53±17 [16-88]	53±17[16-93]	55±16[20-91]	53±17[16-93]
Schooling				
- Basic	137 (33%)	73 (31%)	27 (18%)	237 (30%)
- Third level	133 (32%)	81 (35%)	59 (40%)	273 (34%)
- Secondary	123 (29%)	70 (30%)	55 (37%)	248 (31%)
- No studies	25 (6%)	10 (4%)	6 (4%)	41 (5%)
Family status				
- Father/mother	237 (58%)	114 (50%)	54 (49%)	405 (51%)
- Husband / wife	56 (14%)	59 (26%)	21 (19%)	136 (17%)
- Son / daughter	38 (9%)	17 (8%)	8 (7%)	63 (8%)
- Service	37 (9%)	21 (9%)	15 (13%)	73 (9%)
- Grandfather	23 (6%)	12 (5%)	7 (6%)	42 (5%)
- Single	15 (4%)	3 (1%)	5 (5%)	23 (3%)
- Other (visitor, sharing house)	6 (1%)	0	1 (1%)	7 (1%)

Table 16 – Impact of tiger mosquito on peoples' health.

Year Number of interviews	2008 n=428	2010 n=147	Total n=575
1. Number of householders with recent sting	312 (82%)	119 (89%)	431 (84%)
2. Arthropod responsible for the sting			
- Tiger mosquito (*Ae. Albopictus*)	295 (95%)	94 (79%)	389 (90%)
- Common mosquito (*Culex pipiens*)	7 (2%)	8 (7%)	15 (3%)
- Bee	4 (1%)	0	4 (1%)
- Arachnid	2 (1%)	1 (1%)	4 (1%)
- Others (Wasp, flea)	4 (1)	2 (2%)	4 (1%)
- Unknown	-	15 (13%)	15 (3%)
3. Mean number of stings (only tiger mosquito sting)	6±4[1-50]	4±3[1-20]	5±4[1-50]
4. Location of the sting			
- Legs	122 (41%)	84 (89%)	206 (53%)
- Arms	21 (7%)	35 (37%)	56 (14%)
- Trunk	10 (3%)	13 (14%)	23 (6%)
- Head	12 (4%)	13 (14%)	25 (6%)
5. Lesion type			
- Small swelling (Induration)	211 (73%)	50 (54%)	261 (68%)
- Redness (Erytherm)	48 (17%)	20 (22%)	68 (18%)
- Blistera	21 (7%)	21(23%)	42 (11%)
- Haemorrhage	6 (2%)	1 (1%)	7 (2%)
- Others	4 (1%)	0	4 (1%)
6. Symptom			
- Mild or moderate itching	90 (24%)	24 (21%)	114 (25%)
- Severe itching or pain	254 (74%)	92 (79%)	346 (75%)
6. Sting infection	56 (19%)	22 (24%)	78 (20%)
7. Place of Assistance			
- Primary Care Centre	42 (15%)	19 (22%)	61 (16%)
- Pharmacy	58 (20%)	10 (11%)	68 (18%)
- Private Medical	12 (4%)	3 (3%)	15 (4%)
- Hospital Emergency (Triage)	12 (4%)	7 (8%)	19 (5%)
- Not consulted	165 (57%)	48 (55%)	213 (57%)
I.8. Receive treatment	111 (87%)	28 (57%)	139 (79%)
I.9. Type of treatment:			
- Hydrations (creams)	35 (31%)	2 (7%)	37 (27%)
- Corticoids	19 (17%)	9 (32%)	28 (20%)
- Antihistaminic	23 (21%)	5 (18%)	28 (20%)
- Antibiotic	6 (5%)	4 (14%)	10 (7%)
- Antiseptic	5 (5%)	-	5 (4%)
- Others (anti-inflammatory repellents, aloe vera)	11 (10%)	3 (11%)	14 (10%)
- Unknown	13 (32%)	14 (50%)	27 (19%)

Table 16 - Continued

I.10. Self-administered treatment	154 (54%)	37 (43%)	191 (51%)
I.11. Type of Self-administered treatment			
- Antiseptic (yodo, alcohol, ammonia)	60 (39%)	9 (24%)	69 (36%)
- Hydrations (creams)	36 (23%)	3 (8%)	39 (20%)
- Natural *	33(21%)	5 (14%)	38 (20%)
- Corticoids	4 (3%)	4 (11%)	8 (4%)
- Antihistaminic (D04A)	14 (9%)	6 (16%)	20 (10%)
- Antibiotic	0	0	0 (0%)
- Anti-inflammatory	0	1 (3%)	1 (1%)
- Unknown	4 (3%)	4 (11%)	8 (4%)
- Others (toothpaste, cold water, salty water, homeopathy, saliva)	15(10%)	2 (5%)	17 (9%)

*Natural: vinegar, calamina, lemon, mod, garlic, Aloe vera, tea tree essential oil, eucaliptos essential oil.

Table 17 - Impact on quality of life of the affected population. Knowledge about tiger mosquito and control measures.

Year – Total number of interviews. Householder opinion about tiger mosquito presence.		2008 n=428	2009 n=245	2010 n=147	Total n=820
Section I. Impact on quality of life of the affected population					
I.1. Had problems with tiger mosquito presence at the moment of the interview.		169 (40%)	115 (50%)	100 (69%)	385 (48%)
I.2. Problems with tiger mosquito presence in previous years		346 (83%)	174 (74%)	116 (87%)	636 (81%)
I.3. Consider tiger mosquito a problem		271 (71%)	209 (89%)	122 (84%)	602 (79%)
I.4. Other problematic insects in the householder's opinion	- Culex pipiens	51 (12%)	47 (19%)	8 (5%)	106 (13%)
	- Simulium	10 (2%)	32 (13%)	11 (7%)	53 (6%)
	- Bees	27 (6%)	20 (8%)	5 (10%)	52 (6%)
	- Wasps	18 (4%)	26 (11%)	11 (7%)	55 (7%)
	- Ants	9 (2%)	7 (3%)	6 (4%)	22 (3%)
	- Caterpillars	11 (2%)	2 (1%)	0	12 (1%)
	- Others	15 (3%)	0	11 (6%)	26 (3%)
	- None (Only 2010)	-	-	97 (66%)	97 (1%)
I.5. Consider their quality of life changed by tiger mosquito presence		210 (47%)	122 (46%)	65 (44%)	397 (46%)
I.6. Aspects of quality of life changes:					
- Use of garden		158 (37%)	102 (42%)	57 (39%)	317 (39%)
- Use of the pool		31 (7%)	27 (11%)	9 (6%)	67 (9%)
- Changes in the way of dressing		94 (22%)	28 (11%)	31 (21%)	153 (19%)
- Changes in the home		39 (9%)	17 (7%)	11 (7%)	67 (8%)
- Changes in social life		34 (8%)	23 (9%)	7 (5%)	64 (8%)
- Always has repellents applied		9 (2%)	2 (1%)	7 (5%)	19 (2%)
- Other changes		5 (1%)	4 (2%)	7 (5%)	16 (2%)

Table 17 - Continued

I.7. Number of people affected at home (by stings). Median (range)	2 [0-23]	2 [0-30]	2 [0-15]	2 [0-30]
I.8. Number of people at home	3 [0-30]	3 [1-40]	3 [1-15]	3 [0-40]
I.9. Number of householders who believe that somebody at the house is more affected than others	251 (59%)	-	75 (51%)	326 (56%)
I.10. Characteristics of the person most affected				
- Woman	175 (70%)	-	46 (61%)	221 (68%)
- Man	76 (30%)		29 (29%)	105 (32%)
- Age	40± 24[0-92]		40±26[0-82]	40±25[0-92]
Section II. Knowledge about tiger mosquito and its prevention				
II.1. Already heard about tiger mosquito	418 (98%)	237 (96%)	145 (98%)	800 (97%)
II.2 Already have information about the prevention	373 (87%)	212 (91%)	122 (83%)	707 (87%)
II.3. Media information sources				
- Information leaflets	206 (48%)	128 (52%)	21 (82%)	355 (43%)
- Press, radio and television	191 (45%)	124 (50%)	17 (12%)	332 (40%)
- Local Council (Our informers)	96(22%)	89 (38%)	86 (58%)	281 (33%)
- Health service personnel	32 (7%)	22 (9%)	4 (3%)	58 (7%)
- Word of mouth (neighbours)	24 (6%)	29 (12%)	26 (18%)	79 (10%)
- Others	8 (2%)	1 (0)	8 (5%)	17 (2%)
II.4. Consider more information necessary	246 (61%)	160 (70%)	70 (50%)	476 (61%)
II.5. Has seen a tiger mosquito	342 (5%)	197 (86%)	119 (93%)	658 (86%)
II.6. Knows where are the mosquito breeding spots	366 (88%)	217 (89%)	113 (79%)	696 (87%)
II.7. Takes preventive measures at home.	355 (83%)	209 (85%)	118 (81%)	682 (83%)
II.8. Measures taken				
- Avoid having stagnant water	250 (58%)	141 (57%)	101 (69%)	493 (60%)
- Repellents	196 (46%)	115 (47%)	22 (15%)	333 (41%)
- Insecticide spray	70 (16%)	42 (17%)	9 (6%)	121 (15%)
- Physical barriers	89 (21%)	38 (15%)	10 (7%)	137 (17%)
- Others (candles or natural repellents, homeopathy and use of larvicides)	38 (9%)	24 (10%)	3 (2%)	65 (8%)

With regard to knowledge about how the tiger mosquito reproduces and about related prevention measures, 87% of interviewed persons affirmed that they have this knowledge. Yet, the civil workers after the interview estimated this number to be 76%, based on their observation of the number of households which had not undergone changes during the 3 years.

About 84% of respondents had experienced some insect sting (five on average) before the visit; 90% of these claimed that it was by tiger mosquito. The most frequent type of lesion were small swelling (68%, n=261). As for the most common location of the bites, 53% answered it was the legs (n=206). About 20% of the stings were complicated by infection (n=78). 36% received pharmacological treatment (n=139) and 53% applied various methods of self-treatment (n=191), including self-medication.

Regarding the effects of the mosquito on everyday life, 47% of respondents had problems with the tiger mosquito at the time of the interview (n=385) and 78% had had problems in previous years (n=636).

Within the households which had some larval focus detected during the inspections, 23% more of their householders claimed to have problems with tiger mosquito nuisance (IC 95%: 5–41%) when answering the survey.

80% had seen the tiger mosquito (n=658) and 73% considered it to be a problem (n=602), while there was less concern about other insects. About 46% believed that the tiger mosquito affected their lifestyle (n = 397). Specifically, 48% said they had changed the use of a garden and /or swimming pool (n = 384), 19% had altered their clothing (n=153), 8% their social life (n = 64), 8 % had made changes at home (n=67) and others. A total of 326 people found the tiger mosquito had a preference for some person in the house, mainly a woman (68%) with an average age of 40 years.

An association was observed between the studied year and preventive measures. In 2010 10% more householders avoided the stagnation of water at their dwellings compared to 2008 (CI 95%: 1-19%). Also in 2010 adulticide-spray treatments were implemented in 10% less dwellings compared to 2008 (CI 95%: 5-15%).

An association between the perception of decreasing quality of life caused by the Asian tiger mosquito and level of schooling of the population was observed. Among the population with upper or middle schooling level, 8% more people felt that their quality of life had declined due to the tiger mosquito (CI 95%: 1-15%) than that among the population with basic or no schooling. In no case variations in this perception were observed over the years of study.

58% of those interviewed considered it important to get more information about the Asian tiger mosquito, but there was an association between this variable and the socioeconomic status. Among the population of low socioeconomic status 16% more people declared that they considered it important to have information about prevention measures (CI 95%: 5-26%) than among middle or upper level.

In 2010, 32% of respondents helped by pointing out empty houses in the area (n=36). About 59% of residents knew of a breeding source in the neighbourhood (n=88), 41% said that the focus was in a home property, while 29% believed that the focus was in a stream bed.

The answers about who should be responsible for solving the problem were varied, but 50% answered that it should be solved by everyone collectively, each in their area (n=64) and 41% believed it should be only in the hands of administration (n=45).

4.5. Discussion

The present study brings the first evidence in Europe of the effectiveness of IVM for the control of the tiger mosquito. At the present, there have been only a few examples of successful *Aedes spp*. control (Paupy *et al*. 2009, Kittayapong *et al*. 2008, Jardina *et al*., 1990) and until now the only achievement has been a transitory density control. Moreover, the study also showed how source reduction campaigns based on community participation are a key for a sustainable control of the tiger mosquito and ultimately an important tool to decrease potential risks of diseases (Sánchez *et al.* 2009, Pérez *et al.* 2007).

Integrated vector management techniques have been shown to produce the optimal control strategy (Alphey *et al.* 2010; Ballenger-Browning and Elder 2009). This methodology, where actions such as source reduction, public education, pesticide application, and biological control are combined, has been substituting vertical vector-control programmes, which work with a top-down decision making often exclusively based on chemical interventions.

Worldwide there have been several programmes for *Ae. albopictus* (Wheeler *et al.* 2009, Jardina 1990) and for *Ae. aegypti* control (Arunachalam *et al*. 2010, Vanlerberghe *et al.* 2009, Nan *et al*., 1998, Gubler 1989, Chadee 1988), and initial studies have been directed towards the more ambitious goal of eradication.

However, the results obtained have been described a 'global disaster' due to the spectacular geographical expansion of the insect (Enserink 2008, Kay and Nan 2005). The majority of programmes for *Aedes spp.* control have been focused mainly on *Ae. aegypti*, but both species have their own peculiarities, with consequences for the most suitable control strategies. (Esu *et al.* 2010, Wan *et al.* 2009, Delaunay *et al*. 2009, Heintze and Kroeger 2007, Gratz 2004).

Monitoring by means of ovitraps constitutes an economically sustainable method to estimate the mosquito population density in a risk assessment plan (Carrieri *et. al.* 2008, Focks 2003). The number of eggs represents indirect indices of adult's abundance and provides information on the spatial/temporal distribution of the species. (Roiz *et al.* 2008, Facchinelli *et al.* 2007).

It is a practical method accepted and used to monitor population of *Aedes spp.* adult mosquitoes in the environment (Wan *et al.* 2009; Focks 2003). This method provides several advantages over other methods, ovitraps are inexpensive and sensitive (Carrieri *et al.* 2011a), and can detect the presence of the insect even at low densities (Richards *et al.* 2006). Also it is possible to install them in large areas relatively quickly, bringing ease of field management, achievable even by unskilled staff (Bellini *et. al*.1996).

Despite being successfully used to monitor the impact of various types of control measures involving source reduction and insecticides, there are some theoretical controversies about the use of ovitrap data for assessing adult abundance in high densities (Focks, 2003). However, ovitrap data have been successfully used to monitor the impact of various types of control measures in Italy (Carrieri *et al*. 2006, 2008, 2011; Albieri *et al.* 2010, Regis 2008). Also in 2009 Wan Norafikah *et al*. applied the ovitrap index as an indicator of a degree of infestation in specified areas (Wan *et al.* 2009).

With the aim to characterize the parameters related to a high risk of exposure, data of environmental characteristics and types of premises associated with mosquito breeding sites were obtained, as well as data on the preferred larval breeding sites of the species. These data is crucial for predicting the risk of introduction of vector borne diseases to non-infected areas (Wilder-Smith *et al.* 2012).

Some environmental characteristics found in the premises were associated with the detection of larval breeding spots and adult mosquitoes. Factors as the presence of vegetable gardens, coops and stacked elements in the garden (material for building works, solid waste and firewood) have been shown to be related with the detection of mosquito breeding spots as well as the presence of adults. Such characteristics categorize high risk level of exposure to the tiger mosquito. The finds coincide with another study, carried out in Thailand with the *Ae. aegypti* (Thammapalo *et al.* 2008).

Moreover, specific types of premises were also associated with the detection of mosquito breeding spots and adults. Commercial premises, building working sites and schools were frequently infested. Commercial premises have also been associated with mosquito production in the study of Thammapalo *et al.* (2008). It is worth noting that most of the inspected shops were gardening supply stores (garden centers), thus, several holding water containers were found in their outdoor areas. Schools were also showed to have high risk of exposure, which could be a matter of concern to the public health authorities, as in endemic countries children carry the main burden of morbidity and mortality caused by DEN (WHO 2009).

During the house-to house surveys, most of the visited houses were the first home of the family, the dwellings had outdoor areas with gardens and courtyards. A high percentage of opened containers with standing water were found in the dwellings, without a change in the three years. Flower pot saucers followed by garbage and other small objects like toys were frequently detected in the inspections.

Also the majority of larval habitats and mosquitoes in aerial phase were found in the first family homes. Larval growth was found mostly in spots such as pools, domestic drains (scuppers) and drums with stagnant rain water. In 2012 it was detected that the majority of inspected sites with a larval breeding container also had some mosquito flying around the garden.

In 2010 the study was complemented with larval growth monitoring, *Ae. albopictus* and *Cx. pipiens* were frequently found in the surveys. The tiger mosquito preferred small containers for breeding, spots like scuppers, drums, solid waste and buckets were normally found with *Ae. albopictus* larval growth. Pet water bowls and flower pot saucers (usually with a volume ≤0.5 l) were the most productive ones, displaying higher densities.

On the other hand, no larvae of the tiger mosquito were detected in out of use swimming pools or in temporary pools. In those cases, the common house mosquito (*Cx. pipiens*) larvae were frequently found. Furthermore, the Asian tiger mosquito was found in more premises, in a greater number of containers and with more density than the *Cx. pipens*. This finding could suggest that this invasive species is displacing *Cx pipiens* of some of their habitats, which was observed in another study (Costanzo *et al.* 2005).

The Stegomya indices, Breteau index (BI), container index (CI), house index (HI) obtained in this study were high, BI was 41% (95% CI: 32 -49%), HI was 31% (95% CI: 23-38%) and CI was 9% (95% CI: 7-12%). These results are comparable to indices found in studies carried out in some Asian countries (during 2006-2009), where DEN is endemic (Arunachalam *et al.* 2010). Also using the method of Strickman & Kittayapong (2002) it was observed that the study site was highly infested. It was decided to use those indices because are the most frequent entomological parameters used to measure the risk of dengue transmission in developing countries (Erlanger *et al*. 2008, Silver 2008).

Several years after its first detection, the tiger mosquito was firmly established in the studied area, with high Stegomyia and larval indices, causing immediate nuisance and increasing the risk of epidemics such as DEN and CHIK. Traditional larval indices have been used since the beginning of the 20th century as epidemiologic indicators of dengue transmission in endemic regions, however they are increasingly being seen as inadequate to measure either the risk of transmission or the effectiveness of control strategies, as they do not provide information on the real productivity of containers and their relative contribution to the adult population size (Carrieri *et al*. 2012, Focks 2003).

Furthermore, these indices may be not so useful when implemented as methodology of surveillance in European urban areas due to some basic factors: The different socioeconomic and environmental characteristics of the region and because they they were initially developed for *Aedes aegypti* (Carrieri *et al*. 2011a). In this study the use of those indices has been a complementary method for surveillance, and has been useful to confirm that the area was highly infested.

During the surveys, it was observed that most householders were well informed; they were collaborative and interested in correcting situations in their gardens that could represent a risk for mosquito breeding or sheltering. As a characteristic of the area, there was a high percentage of the population with secondary and third level schooling. This fact could influence their cooperation in adopting previous measures.

Being a key factor for success, the cooperation of the community was requested repeatedly during the door-to-door interventions. The majority of the householders gave access to their property, allowing civil agents to eliminate breeding points in the gardens and back yards. Once they had been informed of the prevention measures most citizens cooperated in the detection of potential breeding points in the areas surrounding their property. Other studies worldwide also suggested positive effects of community based dengue control programmes combined with other vector control methods in zones affected by *Ae. aegypti*. (Vanlerberghe *et al*. 2009, Heitze *et al*. 2007, Kay and Nan 2005).

Householders claimed to have suffered a mean number of five stings the last time they had a mosquito sting. The nuisance of the frequent stings is a problem for the local population; it affects directly the time spent outdoors and their social life, ultimately it has changed their lifestyle. Similar opinions were obtained in a study carried out in Italy, where a high percentage of interviewed householders declared a reduction in outdoor activities and changes of customs in infested areas (Carrieri *et al.* 2008). In that same study the number of stings considered as intolerable by the interviewed people was on average 5 per day.

Frequently the stings resulted in indurations particularly on the limbs; the symptom mostly described was an extreme itch according to interviews with the owners during surveys of the premises. Consistent with other studies (Curcó *et al*., 2008, Giménez *et al*., 2007), about 20% were complicated by infection. Most people received pharmacological treatment and applied various methods of self-curing.

As expected the drug most frequently applied was corticoids. Also some people claimed to self-administrate treatments over the years, usually antiseptics like alcohol, iodine or ammoniac solutions which were frequently applied. Natural treatments such as lemon, vinegar and hydrating creams of Aloe vera and camomile were also often used by the population. Self-treatment methods have clear advantages, but also some inconveniences including that all self-administered antihistamines were from the group ATC D04A (WHO, 2010), being topical antihistamines that are not recommended.

Questionnaires showed that in the last year of study nearly all householders knew about the tiger mosquito and most of them had some knowledge about preventive measures. Even though they were ignoring the public health risks related to health, they considered tiger mosquito the most serious pest that they were expose to, only because of the stings. When they were asked how they prevent mosquitoes in their households, in the last year significantly more householders claimed that they eliminated recipients with stagnant water. On the other hand fewer householders answered that they sprayed insecticides.

Although this fact represents a positive outcome in relation to the education received during the campaigns, no significant changes were observed in the number of dwellings that had recipients with standing water, thus it was observed that increased knowledge does not necessarily equate to behavioural change. This issue was similarly observed in other studies (Ballenger-Browning and Elder 2009, Pai *et al*. 2006, Winch *et al*. 2002, Fernandez *et al*. 1998).

Furthermore, it is supposed that if there were more awareness of the species' medical importance as a vector of diseases; possibly more householders would make more efforts to eliminate the mosquitoes' breeding sites. Thus, the level of engagement of the local citizens would probably be higher (Wheeler *et al.* 2009).

A positive fact was observed about behavioural change over the three study years with regard to the maintenance of the vegetation in the dwellings; it is well known that abundant (uncontrolled) vegetation is a shelter for adult mosquitoes and is directly related to mosquito production (Walton *et al.* 2012). In the last year significantly fewer households were detected with abundant vegetation compared to the first one.

Concerning the private properties, our experience shows that the door-to-door campaigns repeated several times during summer significantly reduced the number of eggs in ovitraps compared to control zones. Thus, it is essential to continue campaigning for the prevention and vector control in the municipality.

Nowadays, concerns about environmental contamination and the emergence of vector resistance to insecticides have resulted in a change in policy from outdoor space-spraying to IVM, where the involvement of local communities is a critical element (Erlanger *et al.* 2008, Heitze *et al*. 2007). Community-based interventions tend to be sustainable as they aim to change behaviour and induce social mobilization (Erlanger *et al.* 2008; Pérez *et al.* 2007; Toledo *et al.* 2007, Winch *et al.* 2002).

In this study the IVM strategy with the combination of four interventions was effective in reducing the number of eggs. Furthermore, a decrease was observed in the number of eggs over time: in 2009 fewer eggs were detected in the reintervention areas compared with the intervention ones, and these differences were statistically significant, which suggests that the door-to-door communication programme can have a long-term effect on the behaviour of the population.

4.6. Conclusions

1 - The IVM control strategy was effective in reducing the number of eggs of the tiger mosquito in the intervened areas compared with the control ones. With the IVM, decrease in the number of eggs over longer time was also observed: in 2009 fewer eggs were detected in the reintervention areas compared with the intervention ones, and these differences were statistically significant.

2 - Some environmental characteristics of the peridomestic areas of premises were found to be associated with Aedes albopictus larval breeding and adult presence. Significantly more larval breeding/adults were detected in premises with vegetable gardens, scuppers, coops and stacked elements left in the outdoor zone (solid waste, materials for building or gardening). Among the types of premises, new building works, garden stores and schools had more cases of mosquito detection (larvae /adult).

3 - The larval density surveys highlight small containers (≤0.5 l) as preferred breeding sites for this species. In the peridomestic areas of the inspected premises, containers like scuppers, drums, solid waste and buckets were frequently found with *Aedes* larval breeding.

4 - The majority of the population of the affected area claimed to know about the Asian tiger mosquito and they considered it to be an important issue that diminishes their quality of life and that deserves more attention from the authorities. They estimate that they had a good knowledge about preventive measures and implemented them at their households. However, field workers detected a high percentage of larval foci in their properties. A high level of public cooperation was obtained due to citizens allowing internal inspection of their

properties and providing information about possible breeding points in the neighbourhood.

Chapter 5

Controlling the tiger: An Interdisciplinary standard protocol proposal

5.1. A description of the scene.

Every year a greater area of the Mediterranean basin provides a home to a new species, the tiger mosquito, which is expanding its range as it has perfectly adapted to the urban environment, using small water holding containers of peridomestic sites of private or public places for breeding and becoming an serious nuisance in people's daily life (Paupy *et al.* 2009).

In addition, with the entrance the area of pathogens through infected travellers into (Fontenille *et al.* 2007, Pialoux *et al.* 2007, Sanchez-Seco *et al.* 2009) there is a risk that the tiger mosquito could change its status from an annoying pest to a potential disease transmitter. *Ae. albopictus* is a competent vector of Dengue (DEN) and Chikungunya (CHIK), which have already been recently transmitted in Europe by the vector (See chapter 2).

Europe has potential vulnerability to the entrance of pathogens, as the current scenario shows intensified human trade and mobility, warmer temperatures, more crowded cities and social inequality, which are factors that have been suggested to influence the evolution, transmission and distribution of infectious diseases (Suk *et al.* 2011).

The Mediterranean area colonized by the tiger mosquito is considered as a "predisposed area", which is defined by the World Health Organization (WHO) as a risk area where existing conditions might facilitate the transmission of a vector-borne disease (VBD) to humans, but the respective pathogen has not been detected (WHO 2012). No vaccines are available today for DEN and Chikungunya (CHIK), thus controlling vector densities is a preventive way to decrease the risk of outbreaks (Medlock *et al.* 2012, Braks *et al.* 2011).

The Catalonian area colonized by the tiger mosquito is included in the "Scenario 3" in the recent publication "Guidelines for the surveillance of invasive mosquito in Europe" (ECDC - Guidelines for the surveillance of invasive mosquitoes in Europe - 2012). Scenario 3 corresponds to situation when an Invasive Mosquito Species (IMS) is widely established, which indicates a situation in which the species has already colonized at least several patches or villages, or a large area/town, with an area of more than 25 km^2. This indicates that the local spread has already started and that the IMS population is large enough to pose a high risk of further spread (ECDC - Guidelines for the surveillance of invasive mosquitoes in Europe - 2012).

Thus, the main challenge to researchers and public health authorities is to set up a program with adequate risk assessment, early detection, prevention and control of emerging VBDs. Vector sampling programs are crucial to determine presence/absence and abundance of vectors for risk surveillance, but also pathogen surveillance at the vector level and host is needed. Getting the figures correct is a major challenge warranting a concerted action on an international and local level (Braks 2011).

The recent guidelines published by ECDC "Guidelines for the surveillance of invasive mosquito in Europe" have the main goal to provide tools to affected countries to support the implementation of tailored surveillance for invasive mosquito species of public health relevance. The guidelines also aim at promoting the harmonization of surveillance methods to obtain comparable data. It also includes a cost estimates and suggest adaptations according to the evolution of the epidemiological situation (ECDC - Guidelines for the surveillance of invasive mosquitoes in Europe - 2012).

Administrative mid-level authorities, such as provincial authorities, should be responsible for homogeneity and quality control of the municipal programs. A standard protocol should be developed through an open participatory process aiming for the harmonization of data collection across different regions and countries (ECDC – Guidelines for the surveillance of invasive mosquitoes in Europe – 2012, Braks *et al.* 2011).

Once the population density of *Ae. albopictus* has reached a critical density, autochthonous transmission of arboviruses can no longer be excluded and appropriate proactive measures must be initiated. According with the ECDC guidelines, every affected country potentially exposed to invasive vectors should adopt directives for surveillance and interventions. According to this international agency, the final responsibility should remain with the national authorities, even if that responsibility is then delegated to districts and communities.

Nowadays, basic information on mosquito distribution and abundance does not exist for several countries or locations (See Table 1 and Figure 1); also there is still a poor understanding of vector density, preferred breeding sites, and vectorial capacity of mosquitoes in temperate climates, including in the Mediterranean basin. This data is highly desirable to understand the risk of introduction of vector borne diseases (VBDs) in non-affected areas (Wilder-Smith *et al.* 2012).

Mosquito management requires considerable lead-time. These programs require, at the very least, trained staff, survey maps and tools, vehicles, spray equipment (if necessary), and insecticides. These needs are all cost considerations requiring advance budgeting, which is rather complicated in a time of governmental budget cuts. Before attempting to launch a mosquito management program, those persons who will manage the program should know how much support there is for mosquito control in the municipality. The support of residents, local civil society groups, business people, and elected officials will be needed.

As seeing in previous chapters (chapters 3 and 4), integrated vector management (IVM) is considered a sustainable method for a long term campaign for mosquito control. There is growing evidence that integrated approaches are highly effective (Erlanger *et al.* 2008, Heitze *et al.* 2007). Surveillance and environmental monitoring, source reduction, public health education combined with the use of biological control agents, larvicides and in the last instance adultices may be part of an IVM based program, where the affected population plays a key role.

A vector surveillance and risk assessment program may include the implementation and management of a monitoring system based on georeferenced traps and the development of a specific Geographic Information System (GIS). Knowing the spatial distribution and abundance of containers with immature mosquitoes may help to set up programs to detect "hot spot" areas, and it can provide a basis for evaluating the effectiveness of control procedures. (Richards *et al.* 2008).

In setting up a Geographic Information System (GIS) to support mosquito control services, data are organized in different layers to describe features such as streets, residences, buildings, train stations, schools, construction sites, shopping centres, medical clinics and electoral divisions. Above these base layers other data can be added such as entomological data, case data, virus serotype, enforcement data, demographics, and weather data and so on.

Recently published reviews about *Aedes* control programs have suggested that effective programs may have had two common elements: First, a vertical component, usually governmental, that initiated, planned, and oversaw the programs, and second, a horizontal component, usually householders, who may help execute control measures and permit access to their property. (Ballenger-Browning and Elder 2009, Erlanger *et al.* 2008, Heitze *et al.* 2007). Although community participation is crucial to success, there are no apparent examples of successful efforts initiated solely at the community level (Heitze *et al.* 2007).

To design and implement a vector control protocol in an urban area, it is necessary to know in depth the eco-socio-environmental characteristics of the place and define the ecological plasticity of the target-organism. Many environmental variables, such as the dimensions of the town, the incidence of small premises or quarters with large buildings, the presence of green areas, the degree of maintenance of the premises and the courtyards, and the number of private and public catch basins affect the density and dispersion of *Ae. albopictus* in the urban environment (Carrieri *et al.* 2011a, Arunachalam *et al.* 2010).

During the surveys performed in the three study years carried out in Sant Cugat del Vallès, more than 3700 visits were carried out and 820 questionnaries were answered.

Preferred vector breeding spots risk level of Asian tiger mosquito exposure were assessed (See chapter 4). Also relations among larval growth spots/adult mosquitoes and some environmental characteristics were found. With the results obtained in the three study years, taking into account the key environmental characteristics that may influence mosquito production and following the advices of the "Guidelines for the surveillance of invasive mosquito in Europe" (ECDC 2012) and other scientific and technical literature found in the bibliographic research, a municipal protocol was designed aiming to keep the tiger mosquito density under a threshold of discomfort and ultimately to prevent emergent VBD outbreaks.

5.2. Setting up a protocol: The main tools.

A bibliographic revision was made, the websites of Health authorities (WHO, EMCA, ECDC, CDC - Center of Disease Control and Prevention) were also consulted. A literature survey through the ISI Web of Knowledge, NCBI PubMed and Google Scholar in the search for methodologies of vector surveillance and control implemented in Europe and other temperate regions was conducted. The key words used in the research were: *Aedes albopictus, Mosquito control, Vector Management, Mosquito guidelines, Europe.*

5.2.3. Annual evolution of *Aedes albopictus* population in Sant Cugat del Vallès and Rubi (2006-2010)

From 2006-2010 and 2007-2010 the Catalonian government (Generalitat de Catalunya) respectively monitored oviposition and BG Sentinel-trap (BGS- traps) (Biogents GmbH, Regensburg, Germany) in infested municipalities. During the mosquito season (from April to November) ovitraps and BGS-traps (See chapter 3) were installed and monitored weekly by the technicians of the Generalitat with the aim of obtaining information about the temporal evolution of *Ae. albopictus* populations in the area.

About ten oviposition traps were distributed through the extension of the municipalities of Sant Cugat del Vallès and Rubí and also two adult BGS-traps were located in the cemeteries of these locations, the geographic situation of the traps are shown in Figure 18 (Chapter 4).

The methodology for the ovitraps monitoring was the same as the one described in chapter 4 with the difference that in this cases it was with a weekly sampling. In the BGS-traps, mosquitoes were caught in bags, which were changed weekly. In the laboratory the bags were frozen and mosquitoes were counted. The species of each individual sample station were identified in the laboratory with a stereomicroscope using the appropriate taxonomic keys (Schaffner *et al.* 2001).

5.2.4. Geographic information systems (GIS)

Mosquito larval breeding detected in public or private spaces were georeferenced with Global Positioning System (GPS) and then mapped with Geomedia Professional, including Geomedia Publisher (Geomedia WebMap Professional).

5.2.5. Legislation

Legislation on vector control agents is probably the most crucial issue to ensure compliance with preventive measures. The Generalitat of Catalonia recently published a model of legislation on the control of *Aedes albopictus* with the objective of municipalities adapting it to their own necessities. The law is very specific for the control of the species, establishing correct ways of maintaining outdoor areas and pools, to prevent mosquito foci.

This model even regulates the ways of storing tires, which is very useful in a prevention campaign. At the time of writing, the Sant Cugat del Vallès legal services use the "municipal ordinance of civil coexistence" to encourage the cooperation of residents or companies. This ordinance regulates the maintainance of public and private outdoor areas under proper conditions of health, safety, hygiene and public use, setting the value of fines at 600€ in case of default.

5.3. The outcomes

5.3.1. Literature review

Several publications focused on mosquito surveillance and control in temperate areas have been accessed to design the protocol. Although there are several research papers about mosquito control and surveillance techniques, currently in Europe there are few scientific publications including Integrated Vector Management (IVM) programs; moreover, no paper with a protocol proposal was encountered. The countries which carry out mosquito surveillance and control programs in Europe are listed in Table 1 (See chapter 2).

From the review of the scientific literature research it was possible to extract relevant data of surveillance control methods implemented in several countries. Key publications about control and monitoring methods are shown in Table 18.

In this research some published guidelines have been found in divulgative (not scientific) journals and webs. Especially in Italy and France, where strategies are proposed by regional governments, there is substantial available information about mosquito control. However, these guidelines have more general information contents; the municipal technicians may need a practical program for mosquito control. The main guideliness found in the research are shown in Table 19.

Practical information on mosquito control and surveillance was achieved in the guidelines published in Italy, Canada, and Switzerland. In Spain, there are some websites from the Mosquito control services with high quality information, crucial for control in the region.

In France there is an organization specially created to control mosquitoes. It is called the EID Atlantique Démoustication Santé, l'Établissement Interdépartemental pour la Démoustication du Littoral Atlantique (In English: The Interdepartmental Institution for Mosquito Control on the the Atlantic Coast).

Table 18 – Key publications of *Aedes spp.* control programs in temperate regions.

Reference		Vector Control Activities: Surveillance monitoring	Control	Source Reduction	Biocides	Disease surveillance	Prediction	Observations
Lindgren *et al.* 2012, Medlock *et al.* 2012; Suk *et al.* 2012; Braks *et al.* 2011; Tilston *et al.* 2009; Hubalek 2008; Farrar *et al.* 2007; Jalinek 2002;	Europe	X				X		Pan-European VBD surveillance
Wilder-Smith *et al.* 2012; Horstick *et al.* 2010; Reiter 2010; Weaver and Reisen 2010; Vanlerberghe *et al.* 2009; Erlanger *et al.* 2009; Ballenger-Browning KK and Elder 2009; Morrisson *et al.* 2008; Farrar *et al.* 2007 Heintze *et al.* 2007; Pialoux *et al.* 2007*	-	X	X	X		X		Dengue/CHIK control and surveillance and reviews
Caminade *et al.* 2012; Semenza and Menne 2009	Europe	X			X	X	X	Pan-European surveillance (Climate change in consideration)
Focks 2003	-	X						Review of sampling methods
Becker et al 2010, Silver 2008, Service 1993	-	X	X	X	X			Sampling and control methods
Paupy *et al.* 2009; Gratz 2004	-							*Aedes albopictus* ecology, vectorial competence
Roiz *et al.* 2007a, *b*	Spain	X						Geographic distribution, larval survey/ ovitraps
Muñoz *et al.* 2011	Barcelona/ Spain	X				X		Host feeding patterns of *Aedes albopictus* and *Culex pipiens*
Eritja *et al.* 2005	Spain	X					X	Geographical distribution, revision.
Valerio *et al.* 2010	Rome/ Italy	X				X		Host feeding patterns of *Aedes albopictus*

Table 18. Continued

Medley 2010	-	X					X	Niche-based distribution, WORLDCLIM and land-cover data
Delauney et al 2009	France	X			X			
Takumi *et al.* 2009, Scholte *et al.* 2008	Netherland	X	X		X			Monitoring and control in green houses
Marini *et al.* 2010	Rome/ Italy	X						Stick traps in mark–release–recapture experiment
Carrieri *et al.* 2012, 2011a-b, 2009, 2008, 2006; Albieri *et al.* 2010	Emilia Romagna/ Italy	X	X	X	X			Ovitraps, larval survey, control
Roiz *et al*. 2010	Trentino/ Italy	X						Effects of temperature and rain fall on the activity and dynamics of *Ae. albopictus.* BGS-traps
Roiz *et al.* 2011; Neteler *et al.* 2011	Trentino/ Italy	X					X	Monitoring (MODIS Land Surface Temperature: LST)
Romi *et al.* 2008, Severini *et al.* 2008	Rome/ Italy	X	X		X			
Romi *et al.* 2006	Rome/ Italy	X	X					Cold acclimation, and overwintering
Kobayashi *et al.* 2002	Japan	X						Larval Survey/ GIS
Bonds. 2012; Hoffman *et al.* 2009; Trout *et al.* 2007;	USA		X		X			Ultra-Low-Volum space sprays (review)
Unlu *et al.* 2011; Bartlett-Healy *et. al* 2011	USA/New Jersey;	X	X	X	X			
Richards et. al 2008; Richards et. al 2006;	USA/North Carolina	X	X	X				
Meeraus *et al*. 2008	Northern Virginia/ USA	X	X					Comparing traps for adult mosquit
Lacey *et al.* 2007	USA		X	X				Control with Bti and B. sphaericus
Pettit *et al.* 2010	Autralia		X		X			Space spraying

Table 18. Continued

Caragata and Walker 2012	Autralia		X	X		The use of bacteria *Wolbachia pipientis*
Racloz *et al.* 2012, Muller *et al.* 2012	Autralia	X			X	Early warning systems
Griffin and Knight 2012	Autralia		X	X		biological larval control with fish
Rapley *et al.* 2009, Willians *et al.* 2006, 2007	Autralia	X				Trapping system

* Systematic revisions, mainly studies in non- temperate regions

Table 19 – Guidelines for mosquito control in temperate regions.

Guidelines	Link
European Centre for Disease Prevention and Control. Guidelines for the surveillance of invasive mosquitoes in Europe. Stockholm: ECDC; 2012.	http://ecdc.europa.eu/en/publications/Publications/TER-Mosquito-surveillance-guidelines.pdf
Estratègia per a la prevenció i el control del mosquit tigre a Catalunya. 2011 (Catalunya – Spain, Regional plan)	http://www.higieneambiental.com/sites/default/files/images/pdf/Estrategia_mosquit_tigre_2011.pdf
Information Web site– Servei de Control de Mosquits del Baix Llobregat	http://www.elbaixllobregat.net/mosquitigre/index.asp
Information Web site– Servei de Control de Mosquits de la Badia de Roses i del Baix Ter	http://www.serveicontrolmosquits.blogspot.com.es/
Municipal Mosquito Control Guidelines 2001 (Canada)	http://wildpro.twycrosszoo.org/S/00Ref/MiscellaneousContents/73MosquitoCanada/Titlepage.htm
Piano di sorveglianza e controllo Chikungunya e Dengue e malattia West Nile anno 2011 (Reggio-Emiglia - Italia, Regional plan)	http://www.saluter.it/documentazione/piani-e-programmi/chik_dengue_westnile_2011.pdf
Piano regionale di monitoraggio della zanzara tigre (*Aedes albopictus*) e la sorveglianza della Chikungunya e Dengue. 2010 (Marche – Italia – Regional plan)	http://www.veterinarialimenti.marche.it/template/1/allegati/zanzara_tigre_presentazione_piano_monitoraggio_2010.pdf
Piano regionale per la lotta alla zanzara tigre e per la prevenzione delle infezioni trasmesse dal vettore. 2008 (Veneto – Italia - Regional plan	http://www.epicentro.iss.it/regioni/veneto/pdf/piano_chiku_Veneto.pdf

Table 19. Continued

Piano de controllo contro la zanzara tigre. 2008 (Umbria – Italia - Regional plan)	http://www.epicentro.iss.it/problemi/zanzara/pdf/zanztigre_piano08-Umbria.pdf
Linee guida per il controllo di Culicidi potenziali vettori di arbivirus in Italia	http://www.iss.it/binary/publ/cont/09_11web.pdf
Per una strategia integrata di lotta alla zanzara tigre. Servizio Sanitario Regionale Venturelli *et al.* 2008 Emilia-Romagna	http://www.zanzaratigreonline.it/Portals/zanzaratigreonline/Materiale_Campagna_2008/Zanzara_Linee%20Guida%202008.pdf
National plan for the control of *Ae. albopictus* Switzerland	http://www.bafu.admin.ch/dokumentation/medieninformation/00962/index.html?lang=de&msg-id=39942
Concept 2011 de lutte contre le moustique tigre (Aedes albopictus) et les maladies qu'il transmet en Suisse	http://www.news.admin.ch/NSBSubscriber/message/attachments/23531.pdf
Plan anti-dissemination du chikungunya et de la dengue (France, local plan)	http://www.ars.paca.sante.fr/fileadmin/PACA/Site_Ars_Paca/Votre_Sante/Veille_sanitaire/Maladies_infectieuses/Chikungunya_et_Dengue/plan_de_lutte_anti_vectorielle/LAV_2011_plan_departemental_definitif.pdf
Guide relatif aux modalités de mise en œuvre du plan anti-dissémination du chikungunya et de la dengue en métropole (France, national plan)	http://circulaire.legifrance.gouv.fr/pdf/2010/05/cir_31164.pdf
EID Atlantique Démoustication Santé – Environnement Établissement Interdépartemental Pour La Démoustication Du Littoral Atlantique	http://www.eidatlantique.eu/page.php?P=116#.
CDC Guidelines for Surveillance, Prevention, & Control. 3th revision. 2003 (Colorado USA)	http://www.cdc.gov/ncidod/dvbid/westnile/resources/wnv-guidelines-aug-2003.pdf

Websites last consulted in September 2012

The biggest and most consulted supranational organizations also have important publications based on scientific studies and expert meetings. The information obtained in these articles was vital for developing a protocol. The main articles are summarized in Table 20.

Table 20 – Key articles of supranational organizations.

Organization	Link
WHO 2012 - International travel and health	http://www.who.int/ith/en/
WHO 2012 - Dengue and severe dengue. Factsheet nº 117	http://www.who.int/mediacentre/factsheets/fs117/en/
WHO 2012 - Chikungunya. Factsheet no. 327	http://www.who.int/mediacentre/factsheets/fs327/en/
WHO 2009 - Dengue: guidelines for diagnosis, treatment, prevention and control WHO/HTM/NTD/DEN/2009.1	http://whqlibdoc.who.int/publications/2009/9789241547871_eng.pdf
WHO 2008 - WHO position statement on integrated vector management. WHO/HTM/NTD/VEM/2008.2	http://whqlibdoc.who.int/hq/2008/WHO_HTM_NTD_VEM_2008.2_eng.pdf
WHO 2007 - Global plan to combat neglected tropical diseases/ WHO/CDS/NTD/2007.3	http://whqlibdoc.who.int/hq/2007/WHO_CDS_NTD_2007.3_eng.pdf
WHO 2006 - Pesticides and their applications for the control of vectors and pests of public health importance. WHO Geneva.WHO/CDS/NTD/WHOPES/GCDPP/2006.1.	http://whqlibdoc.who.int/hq/2006/WHO_CDS_NTD_WHOPES_GCDPP_2006.1_eng.pdf
WHO 2005 - Guidelines for conducting a review of a national dengue prevention and control programme Geneva.WHO/CDS/CPE/PVC/2005.13	http://www.searo.who.int/linkfiles/dengue_bulletins_volumes_29_(2005)_bookreview2.pdf
WHO 2004- Global strategic framework for integrated vector management. Geneva. WHO/CDS/CPE/PVC/2004.10	http://whqlibdoc.who.int/hq/2004/WHO_CDS_CPE_PVC_2004_10.pdf
WHO 2003 - Space Spray Application of Insecticides for Vector and Public Health Pest Control: a Practioner's Guide. Geneva.	
ECDC 2012 - ECDC TECHNICAL REPORT The climatic suitability for dengue transmission in continental Europe	http://ecdc.europa.eu/en/publications/Publications/TER-Climatic-suitablility-dengue.pdf
ECDC/VBORNET project	http://ecdc.europa.eu/en/activities/diseaseprogrammes/emerging_and_vector_borne_diseases/Pages/VBORNET_maps.aspx
2009 ECDC. Development of Aedes albopictus risk maps: Stockholm, ECDC Technical report	http://www.ecdc.europa.eu/en/publications/Publications/0905_TER_Development_of_Aedes_Albopictus_Risk_Maps.pdf

Websites - last consulted September 2012

5.3.2. Annual evolution of *Aedes albopictus* population in Sant Cugat del Vallès and Rubi (2006-2010)

The lowest temperature threshold for seasonal emergence of adults and eggs observed in the five years of study, was an average minimum temperature one week before the first eggs detection in ovitraps of 12.3 ±1.3 [10.0 – 13.2] and 12.6 ± 2.6 [10.4 – 16.4] in BGS-traps. During the years, relative to the start of the season, the first eggs were found between weeks 19 and 24 and the first adults, also with annual variations between week 19 and 28. Moreover, in this area, the end of the annual cycle of the mosquito was observed in ovitraps between weeks 43 to 46 and traps for adults between weeks 43 to 48.

The annual evolution of seasonal abundance of eggs and adults per weeks are depicted in Figure 24 (a-e).

Figure 24 (a-e) Annual evolution of Aedes albopictus population in Sant Cugat del Vallès and Rubi (2006-2010).

Figure 24.a – Year 2006

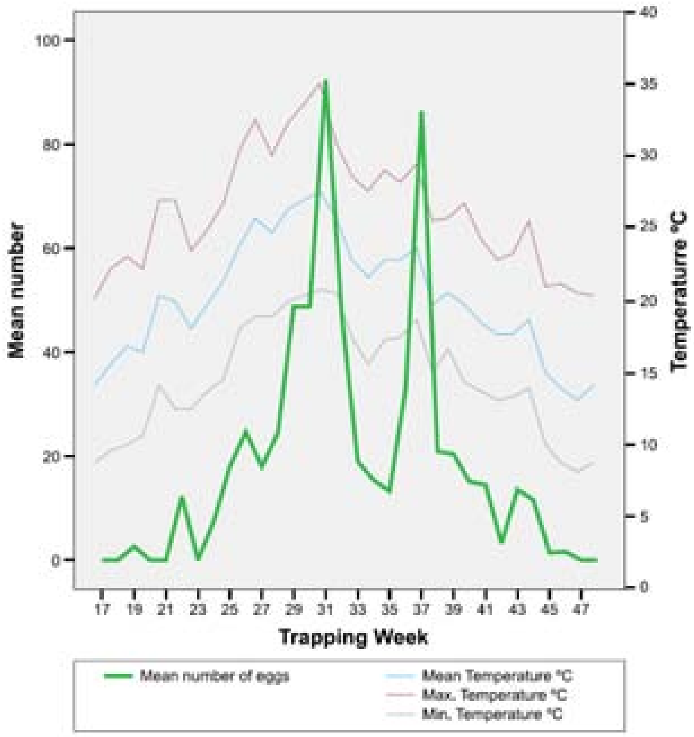


Figure 24.b – Year 2007

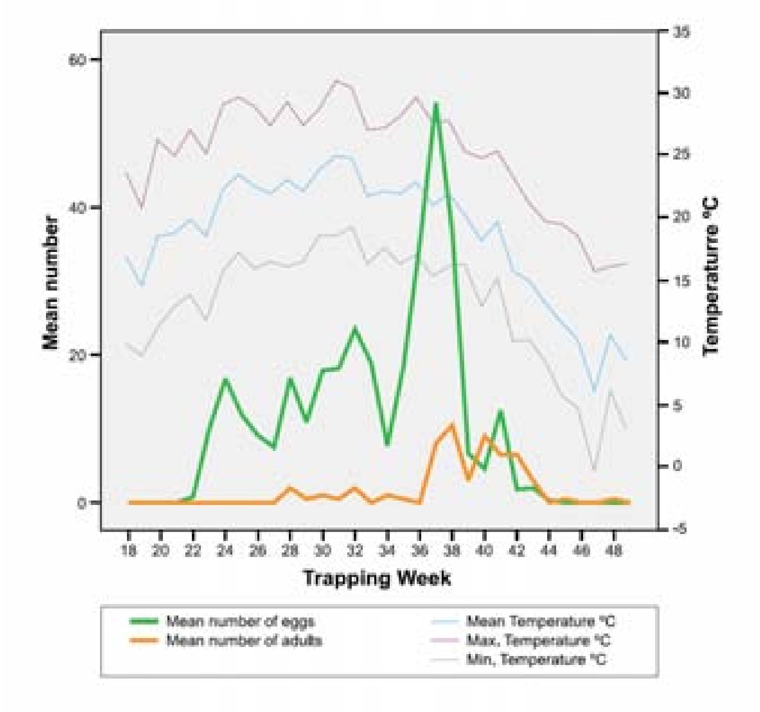


Figure 24.c – Year 2008

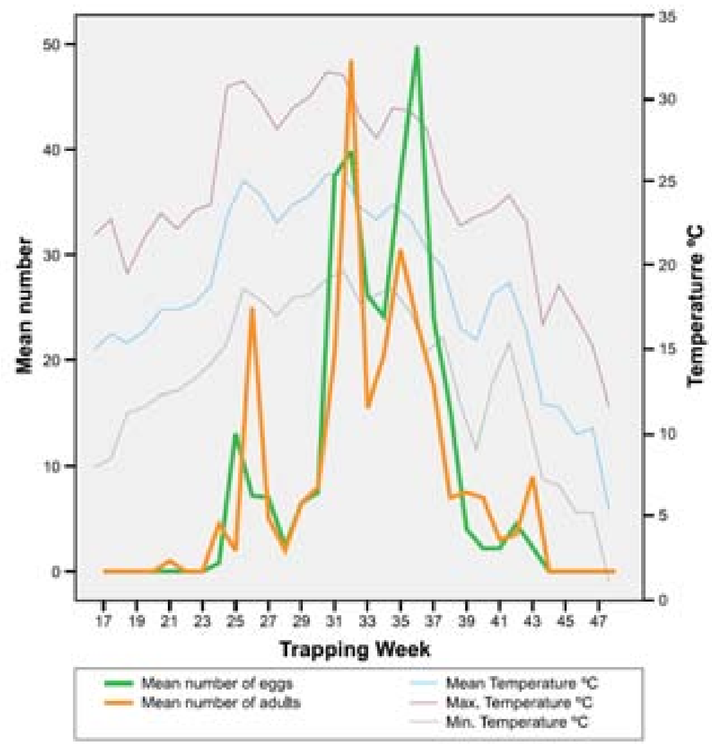


Figure 24.d – Year 2009

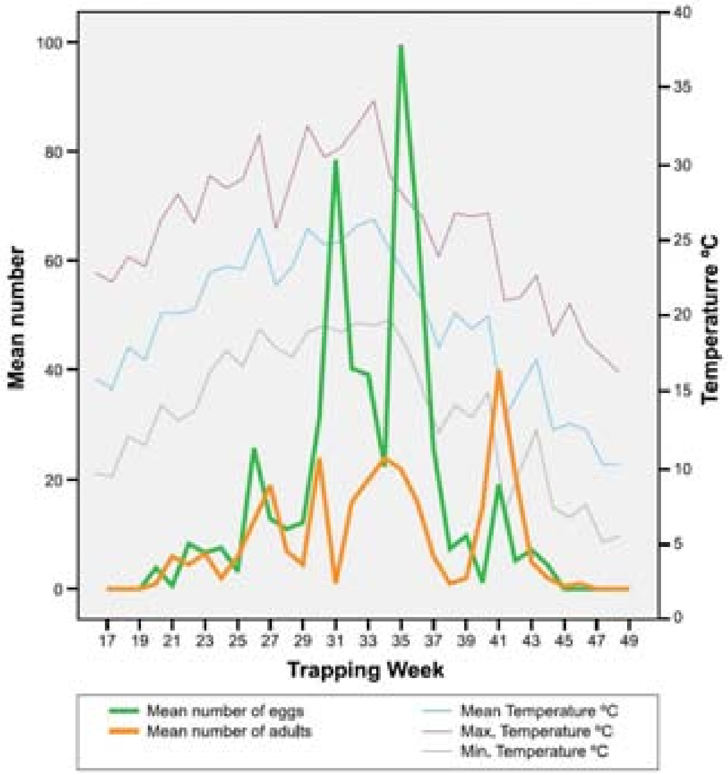
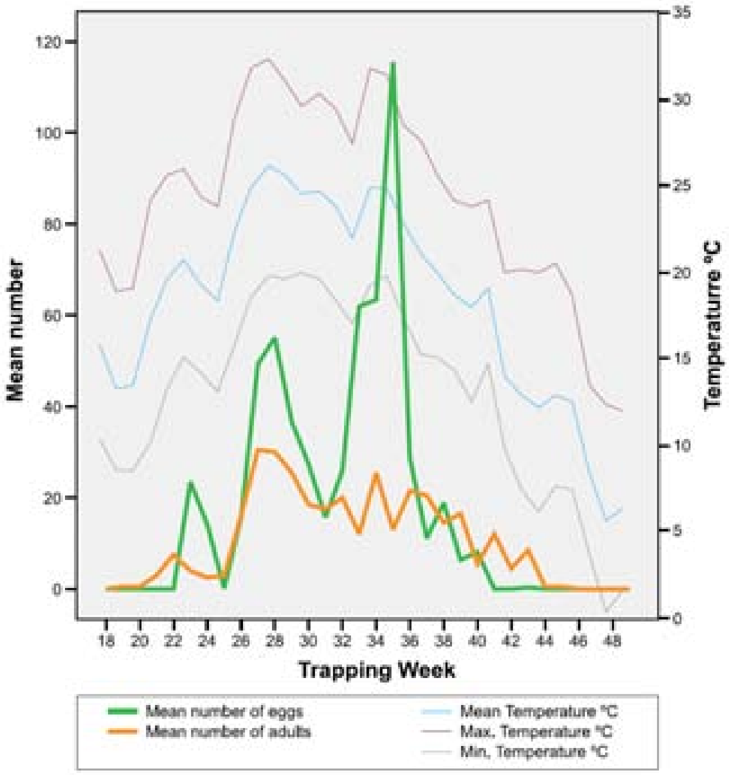


Figure 24.e - Year 2010



5.4. The calendar – The final proposal for Sant Cugat del Vallès.

The official protocol designed and implemented in Sant Cugat del Vallès after eight years of first detection of the tiger mosquito in the area is available in annex 3. The principal measures according to the months of the year for the specific case of Sant Cugat del Vallès are summarized in Table 21.

Integrated vector management includes the following steps:

5.4.1. Educational Outreach Campaign – Communication program

The local Health Authorities may ensure that information about tiger mosquito prevention reaches the vast majority of people in the affected areas to educate and to inform them about domestic water storage and key prevention measures (Vanlerberghe *et al.* 2009, Winch *et al.* 2002). Using different channels of community participation simultaneously is important for community-based vector control (Heintze *et al*. 2007), since it is necessary that people who have breeding points in the households are involved in the campaign.

In Sant Cugat del Vallès from June to September a weekly short article about the tiger mosquito characteristics and peculiarities, including its vector competence, is published in a municipal magazine, which is freely distributed to the population. Also the local television and radio make periodic calls to the householders, inviting them to participate in the control program.

A specific campaign directed at cemetery visitors and plant shops is designed to avoid the use of flowers in water jars, teaching the users other maintenance options for keeping flowers. Also printed information was distributed to pharmacies, where most affected people go to consult (See chapter 4)

The main actions included in the Educational Outreach Campaign are detailed below.

Table 21 - Recommended actions from January to December for an effective integrated vector control strategy for municipalities to control the tiger mosquito.

Actions \ Months	1, 2, 12	3, 4	5	6, 7	8	9, 10	11
Educational Outreach Campaign - community based educational programme							
1 - "Door to door" campaign			■	■	■	■	
2 - Educational campaign in schools			■	■		■	
3 - Conferences and courses to community centres		■	■	■	■	■	■
4 - Communication program		■	■	■	■	■	
5 - Training municipal staff to be actors in the campaign		■	■				
***Aedes albopictus* Surveillance and Control in a municipality**							
6 - Citizen helpline	■	■	■	■	■	■	■
7 - Monitoring of citizens cases	■	■	■	■	■	■	■
8- Installation and monitoring of BGS-traps and ovitraps in the territory*		■	■	■	■	■	
9 - Inspections and possible larvicides treatments, in risk areas		■	■	■	■	■	
10 - Insecticide treatment in case of severe infestation				◆	◆	◆	

* Intervention simultaneously carried out from supra municipal authorities. The black colour of the last line is due to the optionality of this specific intervention

1 - "Door-to-door" (source reduction) campaign

During the hot season, the door-to-door campaign is the key to the efficacy of the Educational campaign. The methodology used in the source reduction campaign is already detailed in chapter 4. The affected population plays a key role in the prevention of mosquito stings and mosquito-borne disease, the contribution concentrates primarily on the removal of breeding points on private properties.

Door–to–door visits may be carried out during the mosquito season, usually from May to October. It was found that it is not as useful to inspect houses in affected areas in the cold season (From November to April in Catalonia) because the dwellings are not yet prepared for the hot season and householders are not interested in receiving information, resulting in misuse of human resources of the civil workers (Bartlett-Healy *et al.* 2011).

Concerning the profile of the civil worker, in Sant Cugat, it was anyone having a degree in environmental or social sciences, biology, geography or even psychology. However, the task could be performed by anyone with personal qualities such as good education, good communication skills, an interest in the biological-environmental issues and shown to be an efficient "door-to-door" educator. Also, one thing to be considered is that the positive motivation of the civil worker is highly desirable to improve the results of the intervention, as it affects the motivation of communities (Vanlerberghe *et al.* 2009).

Civil workers received uniform training. Theoretical and practical knowledge is essential to work in the field. Supervised by municipal technicians, civil workers carried out the inspections of the premises, and the surveys. Potential mosquito breeding spots were reported to householders, advising them to take preventive measures.

The number of required civil workers in a zone depends on its extension and number of single houses with private gardens or courtyards. In Sant Cugat del Vallès (See Table 2), good results were obtained when 4 people work 5 hours a day during six months, covering all the hot season, from May to October. Two thirds of the time the team worked outdoors in the "door to door" campaign and a third of the time was spent in the office carrying out tasks such as updating the databases and organizing the paper material of the campaign.

The visual inspections of the premises were more feasible when they had been performed in blocks as better visibility of the back yards of adjacent households is obtained. All the environmental data was collected on inspection sheets (annex 2); households were classified according to their risk level of exposure to the tiger mosquito. A survey was performed to analyse the level of knowledge of householders about the preventive measures; this knowledge may increase with the civil worker's visit.

A second visit was programmed to confirm that the measures were carried out properly, especially at dwellings where a permanent focus had been detected. In Sant Cugat, in the case of a disabled person who possessed permanent breeding sites at home, regular visits were scheduled to help neutralize the focus.

Inspections began in areas where there are most neighbourhood complaints. All the potential and active breeding points should be georeferenced with a GPS device; the most susceptible areas should be mapped and selected randomly for inspection. Printed educational information is delivered during the door to door campaign; well-illustrated leaflets are especially useful, so that the important information can be easily understood by most people, independently of the schooling level or nationality.
It is also useful that civil workers have the skills to carry out larvicide treatments; this intervention is also included in the IVM strategy and may be implemented when natural actions cannot be achieved.

2 - Educational campaign in schools

A practical workshop where school children of different ages have the opportunity to talk about their personal experiences with the tiger mosquito is suggested. The workshop may highlight possible actions that children could perform at home or in their surroundings that directly affect the density of mosquitoes in their houses. The methodology of the workshop was based on environmental education, whose objective is to change behaviour, promoting an active participation of citizens in society.

In the experience of Sant Cugat (September-October 2011), there was a previous intervention where the teacher asked the pupils to find photos about tiger mosquitos with the help of their families. School children and teachers participated in groups in the realization of a mural displayed on different issues of the presence of *Aedes albopictus* in the environment. The next step, a short story was told in order to motivate the children, reasoning that prevention is the solution to the serious problems. Then, teacher and children (aged 9-12) saw the different stages of the insect during a simulation of an inspection carried out in the garden or playground of the same school. They could play the inspectors outdoors, checking all the containers to look for immature stages of the species, which were previously placed in some spots.

Older pupils (aged 12-14) observed samples of immature mosquitoes' in the laboratory, with microscopes or stereoscopes. Back in the classroom the mural was updated and the new concepts of mosquito prevention were included with changes of pictures. The biological cycle and the immature stages were shown with the illustrations previously placed on the mural and the control actions were focused. To finish, a short film was shown. In the story, set after the establishment of the species, two teenagers in an affected house were able to detect and eliminate all the larval focus of their household, with the help of their friends and neighbours.

A second part of the workshop should be carried out just before the end of school year (May-June) with the same classes which participated in beginning of autumn to reinforce and close the campaigns in schools. Thus, school children were encouraged to teach preventive measures to other pupils and in their homes and neighbourhoods.

3. Conferences and courses in community centres

Speeches and conferences should be very illustrated and practical, with exhibitions of samples of the different stages of the tiger mosquito. In the experience of Sant Cugat del Vallès, it was observed that when the neighbours could recognize a larva, they finally realized the importance of emptying standing water containers in theirs houses. It was common to hear from residents that they thought the mosquito larvae were some worms or tadpoles.

Therefore it is necessary to clarify the differences among species and transfer the responsibility for the sanitation of dwelling to householders. Citizen can be assigned to work together with local government, in detecting breeding points in their neighbourhood or as well ensuring that information about the prevention reaches the majority of local residents.

4 - Communication program

Direct registered letters to companies that may have hot spots can be an effective way to ask for their collaboration with the campaign. In Sant Cugat del Vallès it was often observed that enterprises such as construction companies or the ones that handle used tires and gardening stores had a high risk of holding mosquito breeding points. This intervention should be followed by further inspections to certify that measures are taken. Legislation is an important step to ensure compliance of the prevention measures.

Weekly publications about the ecological behaviour of the species and the campaign were performed. Available information in the municipal web as well as conferences in social centres were key actions to keep the citizen attention on this issue.

Printed informative material was also distributed in different places, like pharmacies, municipal premises, schools, so that more people could access to the preventive methods.

5 - Training and involvement of other municipal personnel (Citizen Information, Police, Forest Guards, Social Services and gardening or public works personnel).

Training municipal staff who work mainly in the street (police, gardeners) or those who work directly with householders (social services) is beneficial for the control campaign. Once they have the knowledge of the biology of *Aedes albopictus* and prevention measures, such workers may cooperate in the detection of new foci in public or private premises. Also they can play the role of environmental educators, being capable of helping their families and neighbours in different aspects of avoiding the presence of mosquitoes in their household.

5.4.2. Surveillance and Control of the tiger mosquito

6. Citizen Helpline

In the municipality it is essential that someone receives phone calls about mosquitoes and gives basic advice. When necessary, inspections can be programmed among municipal technicians and citizens.

7. Monitoring of citizens cases (complaints)

The cases are usually complaints about larval foci in neighbourhood sites, or complaints about the high density of mosquitoes. Often these cases arrive to the council as letters or through the web page, as well as by phone calls, personal visits or warnings from other departments. All the cases must receive an answer from the City Council; often one complaint can lead to the detection of important hot spots, sometimes located in the same household from where the complaint has arrived. Data should be collected; a database should be created for future analysis and the hot spots georeferenced with a GPS device (See annex 3).

8. Vector surveillance

Trapping is an important component in surveillance programs and trapping eggs or adult mosquitoes were suggested to be more appropriate ways of locating sites and times where action should be concentrated to prevent or lessen the intensity of disease outbreaks. Therefore, in addition to the traps monitored by the Generalitat de Catalunya, the Sant Cugat City Council opted to install another 10 oviposition traps and 10 BGS-traps in public schools of different zones of the municipality, with fortnightly sampling.

The decision to install the trap in schools was given because schools may be a key target for control (Wilder-Smith *et al.* 2012). On the one hand it was detected that schools had a high risk of exposure to the tiger mosquito (See chapter 4), on the other hand trapping may be part of the environmental education program, where teachers and school children share their experience with the technicians. Besides, monitoring the traps, technicians can watch over an area where many children spend a considerable amount of time.

9. Larvicide treatments

In Sant Cugat del Vallès the onset of mosquitoes (or eggs) is observed about a week after when the daily minimum temperatures reach 12º C. Then the larvicide treatment program may start. In the case of Sant Cugat, previous mapping was accurately done to highlight the permanent larvae growth spots. (See annex 3)
Diflubenzuron is normally applied by qualified personnel in the de-insecticization program, but also Piriproxyfen was used. Main larval habitats in the public spaces consist of scuppers, building works and solid waste, these last two are normally temporary larval foci, but they also need attention. Occasionally it is useful to do some construction work to definitively avoid the stagnation of water. Periodic cleaning of drains in schools and public buildings also proved to be an efficient measure.
In the case where the breeding points are not yet known, or with the appearance of other points, it is crucial to map and georeference them.

10. Adulticide treatment

Combined with other IVM actions, application of adulticides may be restricted to high risk situations of tiger mosquito exposure, and it should be done only by qualified professionals directly on the typical resting sites of *Ae. albopictus* female mosquitoes (WHO 2003).

The monitoring system with traps is crucial to deciding when it is the correct moment to carry out an adulticide treatment. During the study carried out in Sant Cugat del Vallès, in 2008-2009, adulticides were applied in the intervened areas, usually in public parks localized around the centre of the area (see chapter 4). The intervention was performed when the number of adults mosquitoes detected in BG-traps (monitored by the Generalitat de Catalunya) exceeded 40-50 per week, and then environmental authorities of the Generalitat notified the council to implement the adulticide treatment. The critical number of mosquitoes/trap usually coincided with increasing complaints to the workers of the zone. The level of nuisance could also be used as a risk indicator (Carrieri *et al.* 2009).

ULV applications of alfa-cypermethrin were carried out annually in the cemetery and other affected areas. However this intervention in a public space should be only a last resource, when all the other measures cannot decrease the density of tiger mosquitoes in a specific area.

To improve the issue of environmental impact of mosquito control interventions, the use of space-spraying insecticides may be questioned, its use should be limited to particular situations of severe infestation in vulnerable zone (e.g.: schools, hospitals) or in cases of disease outbreaks (WHO 2005). Pyrethroids spraying is particulary dangerous to the aquatic non-target insects, but also terrestrial non-target fauna, including 'beneficial' organisms, pollinators, and organisms responsible for the maintenance of soil structure and fertility can be severely affected (Hartnik *et al.* 2007). Thus, although the introduction of pyrethroid insecticides represented a great advance in disease vector control, future studies should consider other possibilities for adult control.

5.5. Conclusions and remarks on the protocol proposal.

The standard protocol initially designed for Sant Cugat provides an IVM calendar for the control of *Aedes albopictus* at municipal level in Catalonia, and to the best of our knowledge, is one of the first municipal protocols for mosquito control in the region. Based on the outcomes and analysis after the three studied years in Sant Cugat del Vallès, all the steps for an effective strategy have been listed and detailed so that any municipal technician may understand and implement the protocol.

The strategy evaluated during 2008-2009 in Sant Cugat which included source reduction, larvicide/adulticide treatments and the cleaning of uncontrolled landfills was shown to be effective. Monitored with oviposition traps, it was found that the number of eggs significantly decreased in the treated areas when compared to control ones (See chapter 4 and Abramides *et al*. 2011).

The results of this study, including the several environmental variables that affect mosquito abundance, may be extrmely useful when municipal technicians are designing source reduction and general inspections campaigns. Schools, garden stores and building sites should be on the list of the priority premises to be visited.

Shops that deal with tires must be included in the list. Specific letters may be sent to those centres to emphasize the importance of their collaboration.

During our research some published guidelines have been found in divulgative (not scientific) journals and webs. Especially in Italy and France, where strategies are proposed by regional governments, there is substantial available information about mosquito control. But those guidelines often remain at a quite general level, which may be insufficient for themunicipal technicians who need more practical guidance to develop and implement mosquito control strategies.

The protocol proposed here has however several limitations which are worth mentioning. A serious problem is the lack of information about virus infection. Nowadays the specimens collected in the monitoring systems are not analysed for virus detection, thus, in the case of the introduction of a pathogen, the information may not reach the authorities through this program. Moreover, this limitation is closely linked to the fact that supramunicipal governments have not yet developed a standard strategy towards an effective vector control.

Lack of political will, funding and human resources are often amongst the main limitations to implementing effective municipal control measures. Decision-making on vector control actions in the municipality requires criteria that are relevant to the local eco-epidemiological setting and the inclusion of those control measures that can be locally applied.

In conclusion the protocol presented in this study is a contribution towards an integrated strategy for control and surveillance of the Asian tiger mosquito in the Mediterranean areas.

Chapter 6

Achievements, practical implications and avenues for future research

This study aimed at improving understanding and enhancing knowledge towards an integrated strategy for surveillance and control of the vector *Aedes albopictus* in a temperate area. The findings of this work are relevant in the context of vector control, as they show that a combination of actions can be effective in decreasing vector density. The study provides some insight and practical tools for public environmental/health services and the scientific community.

The arrival of the tiger mosquito to temperate zones opened the way for the entrance of emerging vector-borne diseases (VBDs). Both Dengue and Chikungunya viruses have already been transmitted in the Mediterranean basin, where the mild winter temperatures allow mosquito survival (Paupy *et al.* 2009, Vazeille *et al*. 2008). Nowadays in Europe there concerns and efforts to determine the risk of these diseases becoming endemic are growing (Wilder-Smith *et al.* 2012, Suk *et al*. 2011).

Due to the complexity of understanding the introduction of VBDs, much recent research has focused on some gaps in the analysis. In particular, poor understanding of vector density and preferred breeding sites, and lack of evaluation of current surveillance and control methods of the vector, in particular of the "temperate" Aedes *albopictus,* are issues included in the long list of current research goals (Wilder-Smith *et al*. 2012, Medlock *et al.* 2012).

The objectives of this thesis were:

1 - To evaluate the effectiveness of an Integrated Vector Management (IVM) control strategy of the *Aedes albopictus* population in Sant Cugat del Vallès. The IVM strategy consisted of the combination of four actions: source reduction; larvicide treatments (*Bacillus thuringiensis israelensis* and diflubenzuron); adulticide treatments (alpha-cipermetrin); and cleaning up uncontrolled landfills.

2- To determine several key factors affecting mosquito populations in the premises inspected during the "door-to-door" campaigns (for source reduction)

3 - To identify describe the larval containers preferred by the species.

4- To explore the perception and the involvement of the population of Sant Cugat in the control of this invasive species.

5- To propose a standard protocol towards an IVM control strategy at local level.

In the following we summarise what has been achieved with respect to these objectives. We then discuss our findings in terms of their contribution to science and to their value to health authorities, and identify some shortcomings and limitations, potential practical implications, and potentially interesting lines of future research.

6.1. Achievements of the thesis

With regard to the evaluation of the combined intervention, the median number of eggs in the ovitraps decreased significantly in the treated zones compared to the control ones, indicating that the four complementary actions strategy was effective. This was, to our knowledge the first evidence in Europe of the effectiveness of an integrated vector management strategy for the tiger mosquito. Also, fewer eggs were detected in the two re-intervened areas during the second study year when compared to the ones intervened for the first time that year. The finding suggests that the strategy has an effect extending beyond the year of intervention (Abramides *et al*. 2011).

Thousands of visits were carried out during the three study years and the local community was continually invited to participate, as source reduction and health education were the basis of the strategy. A high level of public cooperation was obtained; the neighbours allowed entrance into their dwellings and also indicated breeding points in the neighbourhood. They considered it to be an important issue that affected their lifestyle and that deserved more attention from the authorities.

The risk level of exposure was assessed by examination of the environmental characteristics of the dwellings and their larval breeding containers. Households with vegetable gardens, coops, scuppers and things piled in the gardens such as garbage, firewood, gardening or working materials were significantly associated with larval breeding and the presence of adult mosquitoes. Gardens with abundant vegetation also turned out to belong to the high risk category. In addition, empty houses, gardening stores, used tire shops, and construction sites also had a high percentage of larval habitats. Therefore, these premises were categorized as having a high risk level of exposure and deserving special attention during the campaigns.

Preferred containers were a target during the inspections. Scuppers, drums, solid waste and buckets were frequently found with *Ae.albopictus* larval growth. Small containers (≤0.5 l) were found with higher larval density than larger ones, containers such as pets' water bowls and plant water saucers had the highest densities. These findings may improve the current knowledge of the larval habitat preferences of the insect in Europe. Houses and gardens with the characteristics highlighted above may be a target when designing campaigns.

Traditional larval indices found in Sant Cugat were inside the range of transmission. Although the use of those indices is not sufficient to evaluate *Aedes* spp. population densities (Focks et al 2000), and especially not in Europe (Carrieri *et al*. 2011a), the high values obtained in this study may corroborate the continuous nuisance and effect on the quality of life declared by the householders during the surveys (See Chapter 4).

The strategy of combined interventions, which turned out to be effective, served as the basis for the design of the proposed protocol (See Chapter 5). The outcome of the three years of study carried out in Sant Cugat allowed the creation and implementation of a mosquito control program for that municipality. The main actions that may help reduce mosquito density include outreach educative programs combined with surveillance and control interventions.

In Catalonia, dependence on local funding and occasionally on regional grants turned out to be one limitation to mosquito control The practical implementation of the protocol may be susceptible to budget cuts, causing unexpected changes in the programs, which may disrupt assessments of the interventions.

Requests for mosquito control, complaints about mosquitoes handled by the City Council and also requests for medical care reached a peak in 2006 and 2004 respectively, and showed a progressive decline after that (Abramides et al 2011, Gimenez *et al* 2007). This fact could indicate a better knowledge by citizens of prevention and control methods and a possible progressive reduction in intensity of the sting due to the local immune response. However, some authors point out the danger of this situation, as a decrease in requests for mosquito control by the citizens is often followed by a decrease in resources invested by the public health administration (Carrieri *et al* 2012).

During recent years, IVM strategies including source reduction, larvicide applications, clearance of abandoned landfill and community education were the key elements of the protocol implemented for the control of the tiger mosquito in Sant Cugat del Vallès, where the insect was first reported in 2004. It would be desirable to carry out further to assess the results and the effectiveness obtained in this program in the longer term.

To sum up, an effective strategy for the control of *Aedes albopictus* in a temperate area and a protocol to be used at a municipal level are presented in this study. At the core of the strategy is a combination of vector control activities with educational programmes aiming at increasing the communities' knowledge of potential breeding sites nd its implication in reducing vector densities. Community education, public participation and strong engagement of policy-makers appear as key elements of the strategy to ensure effectiveness of the program over the longer term.

6.2 The view from the "front line" – Personal experience

At the empirical level, this thesis builds on the experience of the author as an agent employed by the municipality of Sant Cugat del Vallès to design and implement a tiger mosquito control strategy.

From the point of view of public administration, specifically at the municipal level, the changes in people's lifestyle brought about by the invasion of the tiger mosquito was a singular experience. Phrases like "we can't stay in the garden anymore", "we must wear long clothes the whole summer" and "our grandchildren do not come to visit us any more" have been often repeated in the hundreds of telephone calls received during recent summers.

More extreme situations have also been reported by neighbours who said they were "living in permanent stress" or "in a state of psychosis", "paranoid" and even "in deep depression" or "prisoners inside the house", due to the presence of this annoying pest. Pensioners complained about spending a high percentage of their salary on products for protection and treatments. One householder explained a peculiar social change in recent summers: when guests came to his house, instead of offering them a drink, he was first offering an insect repellent, antiseptic or even an antihistaminic.

All these stories have been complemented with the clinical cases; children and adults have sought medical assistance in large numbers. While severe itching or pain were the main symptoms and small swelling the most common lesion, several reports of haemorrhage and infections have been documented (Curcó et al 2008, see chapter 4). The experience of one person with a hundred punctures on all parts of the body was reported to the city council.

Furthermore, municipal technicians working in "the front line", giving support to the affected neighbourhoods were astonished to realize that the same people who were suffering with the "tiger's attack" were unintentionally providing breeding spots to the mosquitoes. Larval habitat could be any water holding object in any neighbour's garden or in a public space. For the local administration it became evident that only a combination of proactive measures could change this paradoxical situation.

Street fumigations, the main measure initially requested by neighbours would not work, as the mosquitoes' breeding points were often inside private gardens. Mosquito Control Services, existing since the 80s mainly to control mosquitoes from wetlands, were very helpful in providing information about mosquito ecology and promoting conferences; however the problem had also exceeded their available resources. Action by supra-municipal bodies was mostly limited to distribution of some printed material about the insect, as no direct control intervention was planned at that level at that time.

In the fortunate case of Sant Cugat, policy makers interfaced with a multidisciplinary team of scientists to set up an action plan aimed at performing and evaluating an innovative strategy. As only scarce information on tiger mosquito control was available, international literature, mainly about *Aedes aegytpi* was consulted. Cuba, Brazil, Vietnam, Thailand, Singapore and several other countries had been fighting against the dengue vector for decades, using all kinds of control programs. Community participation, source reduction, health education and intersectorial coordination across different authorities and departments were the key actions suggested to achieve a successful program.

During the implementation of the strategy, entrance in the affected households to control the containers was an important factor to be achieved. However, the removal of potential breeding points, often detected even from outside the fence, or simply reported by a neighbour, could generate a delicate situation for both civil worker and householder.

From the frontline point of view, the primary way to get householders' collaboration was by inviting them to participate in the program: *"Are you affected by mosquito stings? Are there many in this area?"* These questions were appropriated to start with. Residents should never feel threatened by the civic officer's visit, who is there to help them and not to give out fines (Bartlet-Healey *et* *al*. 2011). The motto should be "uniting together it is possible to decrease the mosquito nuisance".

With neighbours becoming key protagonists in the campaign, some changes were achieved in people's behaviour. The surveys in the last year indicated that the fumigations in the private gardens have been substituted by simply turning over of containers; also more attention was given to controlling the vegetation.

Being totally the opposite of obsolete vertical programs, where insecticide spraying was the main intervention, this study reflects an effective strategy designed from, and performed for, community needs. It is essential to go out into the field to achieve tiger mosquito control; by just listening to the householders and their communities one can learn a lot about ways to help them against this pest.

6.3. Present and Future trends in Research and Practice

In this study an effective IVM strategy for the control of the Asian tiger mosquito was achieved and based on this study a standard protocol was designed. This protocol is being implemented in the first affected municipality of Catalonia (Annex 3), with possibilities of being adapted to other municipalities.

Nowadays only few affected regions in the Mediterranean basin have some control plan, which is crucial to avoid mosquito nuisance and ultimately mosquito-borne diseases. It is feasible to apply the control strategies and the municipal protocol achieved in this study to other places of Catalonia and in the Mediterranean basin, as the similarities in weather, preferred larval habitats and resting sites have been described (Lambrects *et al*. 2010, Paupy *et al*. 2009).

However, a previous diagnosis should be carried out to detect the main features of the intervened places. These *ex ante* studies may include detection of the potential foci in public places and marking zones where premises with high risk of exposure are more concentrated. Not only ecological but also socioeconomic factors affect vector breeding (Arunachalam *et al.* 2010). In addition, the estimation of minimum temperatures for the beginning of the mosquito season could be part of the diagnosis. With the tools suggested in this study, the setting up process in other Catalonian or Mediterranean municipality may be an easier task.

Nowadays globalization and climate change are leading to variations in the geographic distribution and frequency of vector and VBDs, and Europe is included in the vulnerable zones (Fontenille *et al.* 2007; Vazeille *et al*. 2008). International experts are working to set up an effective surveillance system which include predictions of changes caused by global warming (Caminade *el al.* 2012, Semenza *et al.* 2012).

Supranational health agencies WHO, EMCA, ECDC/VBORNET have been promoting international meetings among medical entomologists and public health experts, aiming at creating an adequate risk assessment. European guidelines have been recently published with the goal of providing several tools for surveillance and control. Lack of funding is still the major limitation to implement a control strategy in many regions.

The findings of our study provide a link between the projects carried out by supranational health bodies and the urgent need for municipalities and communities to achieve some level of control of the tiger mosquito. Scientists and health authorities could implement programs analogous to the one presented here in several places simultaneously, perhaps sharing human and material resources to reduce public spending. Interactions between multiple disciplines are necessary and policy makers are playing an important role in setting up an integrated strategy (Wilder-Smith *et al*. 2012, Braks *et al.* 2011).

Additionally, it may be remarked that bio-environmental education at schools is another essential area to be included in future protocols, as it is a crucial tool that may be implemented (in a vector control campaign) aiming at long lasting benefits. Children necessarily experience their biological environment, thus it is important to motivate them with knowledge to have a personal engagement with this issue, just like any other issue of ecology today. They may influence future decisions and behaviour at many different levels.

To sum up, this study may serve as a bridge between supra national decisions and community necessities. The effective control strategy and protocol proposed in this work could be further adapted to other municipalities in the Mediterranean basin. Previous studies should be performed for adjusting the model to necessities of other affected areas. Further research is needed to assess the effectiveness of the strategy in an expanded area.

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Annex List

Annex 1 – The published article.

Annex 2 – Inspection sheeds, questionnaire and printed letter used in the "door-to-door" campaign.

Annex 3 – The protocol of the IVM control strategy implemented in Sant Cugat del Vallès.

Annex 1

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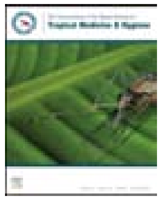

Effectiveness of a multiple intervention strategy for the control of the tiger mosquito (*Aedes albopictus*) in Spain

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ABSTRACT

This study was undertaken to evaluate the effectiveness of four complementary and combined strategies to minimize the presence of the invasive mosquito *Aedes albopictus*, firmly established in Sant Cugat del Vallès, Catalonia, Spain. A quasi-experimental design including six neighbourhoods was performed in 2008–2009. The abundance of mosquitoes was monitored through ovitraps. The multiple intervention strategy consisted of four actions: source reduction; larvicide treatments (*Bacillus thuringiensis israelensis* and diflubenzuron); adulticide treatments (alfacipermetrin); and cleaning up uncontrolled landfills. The results showed the number of eggs significantly reduced in the areas with intervention. In 2008, the accumulate median of eggs was 175 and 272 in the intervention and control areas, respectively. In 2009, these medians were 884 and 1668 eggs. In total, 3104 households were visited and 683 people were interviewed. During inspections inside the houses, the cooperation of citizens in 2009 was 16% higher than that in 2008 (95% CI 13–19%). These findings suggest that the strategy was effective in reducing the number of eggs. Citizen cooperation, an essential factor for success, was observed through a high level of collaboration by the home owners, who allowed entry into their private dwellings. This study could be a model for controlling the populations of *Ae. albopictus* in the Mediterranean region.

1. Introduction

The Asian tiger mosquito *Aedes albopictus* (Skuse 1894) (Diptera: Culicidae) is an invasive species, originally indigenous to the forests of Southeast Asia, that in recent decades has spread to many temperate and tropical regions of the world, including southern Europe.[1–3] It was detected in Spain for the first time in Sant Cugat del Vallès, Catalonia, during the summer of 2004.[4,5] *Aedes albopictus* is a synanthropic and daytime biting species. Its use of artificial containers in suburban landscapes as breeding sites was one of the reasons that contributed to its rapid geographic spread.[3] Nowadays the tiger mosquito has colonized 119 municipalities of Catalonia, affecting potentially approximately 5 million people. In Sant Cugat, *Ae. albopictus* is currently well established, becoming a major pest organism and affecting people's health and their quality of life.[6]

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The tiger mosquito is dangerous, owing to its potential implications for public health.[3,7,8] This species is capable of transmitting many diseases to humans, being an important vector of several arboviruses, such as dengue (DEN), chikungunya (CHIK) virus, yellow fever and several other types of encephalitis.[1,8,9] DEN is the most important arboviral disease in the world, affecting more than 50 million people every year. Despite *Ae. aegypti* being responsible for most cases, *Ae. albopictus* has been associated with some outbreaks of DEN.[1,8] An outbreak of CHIK fever in the summer of 2007 in northeast Italy, with 200 diagnosed cases,[10] was the first confirmation that temperate *Ae. albopictus* populations could transmit a tropical virus imported by travellers and cause an epidemic in a colonized European area, creating an important health concern.

Although a number of control strategies for mosquito-borne diseases operate in different localities and countries, integrated vector management (IVM) techniques, such as source reduction (SR, a community-based approach), pesticide application, biological control, education and public awareness, as well as personal protection seem to offer the most promising results.[7,11–13] An important point in carrying out long-term effective control is that it is necessary to include the collaboration of people who possess domestic points of breeding.[11] Several researchers advocate that SR efforts are the only sustainable way to control *Aedes* (Stegomyia) vectors.[14–17] Data show that once the tiger mosquito is established in a zone it is nearly impossible to eliminate it and very difficult to reduce the size of its population.[18]

The important spread of *Ae. albopictus* through all continents in recent decades,[1,3] the importation of active cases of CHIK and DEN,[8,19] the vectorial capacity for both viruses[20] and the vulnerability of southern Europe to virus introduction[2,21] emphasize the importance of development and application of strategies for IVM control methodologies. The aim of this study was to evaluate the efficiency of four complementary and combined strategies in the minimization of the *Ae. albopictus* population in one of the largest municipalities in Catalonia.

2. Materials and methods

2.1. Study site

The study was performed in Sant Cugat del Vallès (41°28′4″N, 1°53′49″E, 48.32 km^2; mean elevation 172 m) and the nearby municipality of Rubí (41°29′36″N, 02°01′57″E, 32.30 km^2; mean elevation 123 m). Both are residential Catalan towns (population 82 642 and 73 691 inhabitants, respectively) with many parks and large areas of single houses with private gardens, courtyards and pools. The average annual rainfall is 605 mm, and the average minimum temperature is 10.2 °C, with a typical Mediterranean climate. Sant Cugat and Rubí are located at 15 km and 20 km, respectively, northwest of Barcelona, from which both urban areas are biogeographically separated by the Natural Park of Collserola.

The six studied zones are depicted in Figure 1 and Table 1. Areas 1 and 2 had intervention for two consecutive years: 2008 and 2009. Areas 3 and 4 were used as controls during 2008, but after the city council carried out SR programmes in all houses where citizens had complained or asked for technical support, they were included in 2009 as intervention areas. Control zones (areas 5 and 6) were located in Rubí, where the tiger mosquito was detected later and the city council had not promoted any *Aedes* spp. control programmes at the time of the study.

The study areas comprised mainly single-family dwellings and were segregated from surrounding neighbourhoods by large roads, woodlands or building complexes. There were 100 to 470 houses in each neighbourhood, and the mean lot size was 0.17–0.25 ha. All housing and inhabitants in the six study areas were included, and only people who refused to participate in the study, those with mental disabilities and those <16 years were excluded.

2.2. Study design

This was a quasi-experimental study with multiple interventions, carried out from February to October in 2008 and from May to December in 2009, and consisted of four complementary strategies. The first of these was SR. House-to-house visits were carried out in each studied neighbourhood. The field workers asked for permission to enter the properties to educate the citizens about measures to prevent mosquito-borne disease. SR achieved through environmental sanitation of containers was used as a method for experimentally manipulating the production of immature *Ae. albopictus* in container habitats. As many residences as possible within neighbourhoods were surveyed for water-holding containers. In intervention areas, any water remaining in a container was discarded, and the container was turned over so that it would not collect rainwater. Any wet containers that could not be emptied were treated with an insect growth regulator larvicide (diflubenzuron 2% at a concentration of 1 g/hl) (Flower, Lleida, Spain). SR measures were conducted after adult householders gave verbal informed consent. The outcome of each visit was recorded in detail on a form and given to the city council.

The second measure was larvicide treatment with DEVICE TB2 (diflubenzuron 2% at 1 g/hl) in scuppers, water tanks and street drains containing stagnant water in the intervention areas. A granular formulation of the biolarvicide *Bacillus thuringiensis israelensis* (Bti), Vectobac G (EPA Registration No. 73049-10) (1.2% Bti, 1 g/m^2) (Valent BioSciences Corporation, Libertyville, IL, USA), was applied to seasonal streams. The third measure was sanitization of municipal sites and wooded terrains, with removal of uncontrolled rubbish dumps in the intervention zones. The fourth measure was adulticide treatment (Fastac 10% – alfacipermetrin 50 cc/hl) (Basf Española S.A., Tarragona, Spain). Monthly, from July to October 2008–2009, insecticide was sprayed on the vegetation of some public gardens of each neighbourhood study area by specialized teams. These isolated fumigations were carried out, selecting two or three points in each intervention area, and giving priority to public gardens with the greatest number of users, as well as points located centrally in the intervention area to achieve a greater effect.[22]

Table 1
Characteristics of study areas and the combined interventions performed during 2008 and 2009

Intervention		2008					2009						
		Intervention neighbourhoods		Control neighbourhoods		Other areas	Intervention neighbourhoods		Reintervention neighbourhoods		Control neighbourhoods		Other areas
		Area 1 Les Planes	Area 2 Can Cortès	Area 3 Mas Gener	Area 4 Can Barata	Standard	Area 3 Mas Gener	Area 4 Can Barata	Area 1 Les Planes	Area 2 Can Cortès	Area 5 Can Ximelis	Area 6 Can Mir	Standard
	Area ($\times 10^3$ m^2)	80.5	73.3	60.2	67.7	4700	60.2	67.7	80.5	73.3	60.0	60.2	4700
	Number of dwellings	334	100	470	232	24 014	470	232	334	100	150	280	24 014
Source reduction	Visited houses *n* (% total dwellings)[a]	296 (89)	48 (48)	61 (13)	18 (8)	1681 (7)	345 (89)	109 (46)	80 (24)	46 (46)	17 (11)	20 (7)	383 (2)
	Accessibility *n* (%)[b]	145 (49)	33 (69)	39 (64)	13 (72)	926 (55)	174 (50)	61 (56)	25 (31)	13 (28)	14 (82)	14 (70)	281 (73)
	Inspections inside the house *n* (%)[b]	42 (14)	6 (12)	15 (25)	3 (17)	209 (19)	67 (19)	24 (22)	12 (15)	12 (26)	1 (1)	3 (1)	172 (49)
	Houses with water holding containers *n* (%)[b]	152 (51)	21 (43)	32 (52)	10 (55)	709 (42)	186 (54)	52 (48)	31 (39)	19 (41)	12 (70)	18 (90)	211 (52)
	Houses with larval habitats *n* (%)[b]	18 (6)	0	2 (3)	1 (6)	32 (2)	17 (5)	2 (2)	8 (10)	6 (13)	0	0	86 (22)
	Interviews *n* (%)[b]	91 (31)	11 (23)	17 (28)	5 (5)	303 (18)	70 (20)	27 (27)	20 (25)	12 (26)	4 (23)	6 (30)	107 (28)
Waste removal	Area (ha) (%)[c]	0.5 (0.8)	0.1 (0.1)	0.5 (0.8)	0.2 (0.0)	–	1 (1.7)	0.4 (0.6)	0.3 (0.4)	0.3 (0.4)	–	–	–
Adulticide (periodic treatment)	Area (ha) (%)[c]	0.7 (0.9)	0.5 (0.7)	–	–	–	1 (1.7)	0.5 (0.7)	0.5 (0.8)	0.5 (0.7)	–	–	–
Larvicide (periodic treatment)	Number of public sites with larvicide treatment (l)	1 (2)	1 (2)	–	–	–	15 (50)	–	1 (2)	1 (2)	–	–	–

[a] Total of dwellings in the area.
[b] Total of visits in the area.
[c] Total of study area (ha).

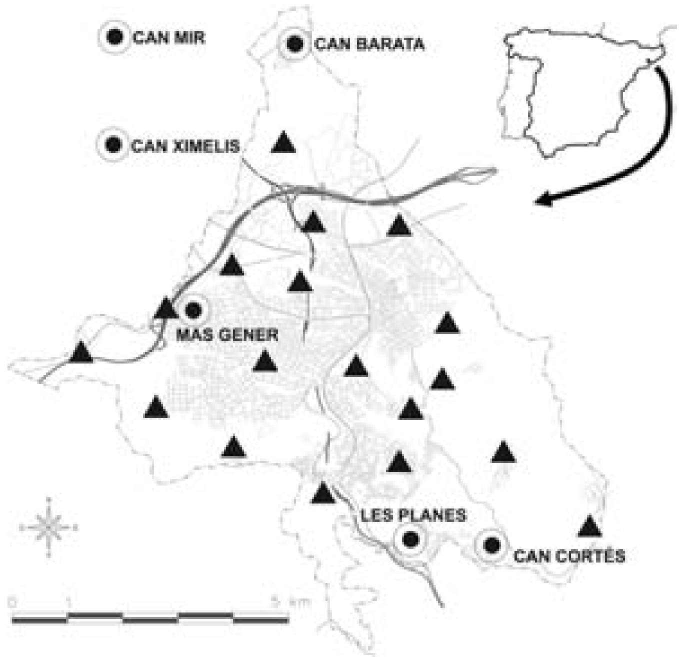

Figure 1. Geographic situation of the zones under study (see Table 1 for more details). The black triangles indicate the locations of oviposition traps in the standard area of the municipality of Sant Cugat del Vallès in 2008.

2.3. Surveys

A household was defined as one separate unit of accommodation (individual home or apartment) and the immediate surrounding premises, irrespective of the number of people residing within the unit. Information about housing type (principal domicile, empty house or service) from each household of the studied zones was collected. Neighbours from the whole municipality of Sant Cugat could also ask for technical support from the city council. All cases reported in this municipality were attended to with standard support: a civil agent inspected the household and SR measures were applied at the affected house and in all the houses that surrounded it; householders were interviewed and data were collected. The area and procedure were defined as standard. Good accessibility was defined as the situation in which the surfaces of the back yard or garden of the household were mostly visible even if the field workers could not enter it. When there was nobody at home at the time of the visit or when the citizens would not collaborate with the civil workers at the time of the inspection, an official letter emphasizing the need of collaboration with written and illustrated advice was sent, explaining how to avoid mosquito reproduction in the dwellings. Data were collected from some houses from control areas, selected randomly, and in those cases the inspections were carried out from outside the dwelling at the end of the season.

2.4. Ovitraps

About 15 oviposition traps (ovitraps) were allocated in each of the six study areas, approximately 200 m one from the other. Each sample station consisted of one ovitrap, a black plastic glass with a diameter of 14 cm filled with 300 ml of clean water and containing a half-immersed piece of wood measuring 2.5×12.5 cm. The ovitraps were placed in sites shaded by vegetation in the treated and untreated neighbourhoods. The paddles were collected fortnightly and placed in individual plastic drawers that were sealed with parafilm and labelled. The water in the ovitrap was always checked for hatched mosquito larvae and/or pupae. Fresh tap water, new paddles and missing ovitraps were systematically replaced. Biolarvicide Bti was applied to prevent the production of mosquitoes in the trap. The number of eggs collected per trap was assessed by examination under a stereomicroscope ($40\times$). All the surfaces, including the edges, were checked and the eggs were counted. Population abundance was expressed by the mean and median number of eggs per positive trap. Because a small portion of the eggs laid (less than 5%) could belong to other tree hole *Aedes* mosquitoes (*Ae. geniculatus*, *Ae. echinus* or *Ae. berlandi*)[23], we raised them in the laboratory based on Roiz et al. (2008) to obtain larvae to confirm the identification of individuals. All the emerged larvae were identified as *Ae. albopictus*. This result, together with the results of other studies in the same area,[24] confirmed that the eggs of the ovitraps were *Ae. albopictus*. The number of eggs represents the abundance of sexually active females.[24] Ovitrap monitoring was performed from August to October in 2008 and from May to December in 2009. The months with greatest activity of *Ae. albopictus* were always studied, but with variation in the follow-up procedures, due to changes in the resources available each year. In 2008, as well as in the study zones, 18 ovitraps were homogeneously distributed through the town, corresponding to the standard municipality area. In 2009, the standard area was not monitored by ovitraps.

2.5. Statistical analysis

Data were analysed with a negative binomial distribution generalized linear model (GLM). Missing data were replaced using the method of the median of adjacent points. The response variable was the egg abundance, the intervention type is an explanatory variable (factor) and month and area were introduced as covariates to control for their possible effect and to centre the analysis on the effect of the different level of the treatments ('control', 'intervention' and 'reintervention'). Analysis was performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results

In total, 2104 houses were visited in 2008, and 1000 in 2009. The main findings in the study areas of the four complementary interventions are shown in Table 1. The number of citizens interviewed by questionnaire was 427 in 2008 and 246 in 2009. The distribution of the questionnaires was 60% in standard areas ($n = 409$), 34% in intervention areas ($n = 230$) and 6% in control areas ($n = 44$). In the intervention areas, 47% of the citizens interviewed (102 questionnaires) allowed the civil workers to enter their dwellings in 2008 and 90% (128 questionnaires) did so in 2009.

At 9% of the visited houses it was not possible to see the back yards and gardens of the dwellings, specifically in 10% ($n = 210$) in 2008 and 6% ($n = 60$) in 2009. Accessibility for years did not show differences in control and intervention areas, but in 2009 the accessibility to standard areas was 18% higher than in 2008 (95% CI 13–23%). During inspections inside the houses, the cooperation of citizens in 2009 was 16% higher than that of 2008 (95% CI 13–19%). About interviews, no differences were observed for years in the control areas, but an increase of 4% was observed in 2009 in intervention and standard areas compared with 2008 (95% CI 1–8%). Also the detection of containers with stagnant water (95% CI 1–13%) and containers with production of immature mosquitoes (95% CI 7–11%) increased by 9% in 2009 compared with 2008.

Regarding the two other interventions in the study areas, larvicide treatment and clearance and waste removal, these were similar during 2008 and 2009, but adulticide treatment was applied four times in 2009 and only once in 2008. Selection of the insecticides used was based on data from recent literature.[11,12,23] In relation to the standard areas, the number of cases reported to the city council was 206 in 2008 and 170 in 2009.

Results showed a significant reduction in numbers of mosquito eggs in treated areas compared with untreated areas in 2008 and 2009 (Figure 2). The median number of eggs was higher in 2009.

Table 2 shows the resulting model after statistical analysis. All three studied variables (intervention, area and month) affected the egg abundance ($P < 0.05$). Control areas show significantly higher egg abundance than the other areas. Intervention areas also presented significantly higher egg abundance than the reintervention areas.

4. Discussion

The present study brings the first evidence in Europe of the effectiveness of IVM for control of the tiger mosquito. At present, there have been only a few examples of successful *Aedes* spp. control,[3,15] and until now the only achievement has been a transitory density control.[16,25] Around the world, the majority of programmes for *Aedes* spp. control have been focused mainly on *Ae. aegypti*, and initial studies have been directed towards the more ambitious goal of eradication.[26–28] However, the results obtained have been considered a 'global disaster'.[29] Both species have their own peculiarities, with consequences for the most suitable control strategies.[1,2,7,11,13]

Currently, the most practical method accepted and used to detect and estimate the population of *Aedes* spp. adult mosquitoes in the environment is the ovitrap.[13,30] The advantages of ovitraps are that they are inexpensive and sensitive, and it is possible to install them in large areas relatively quickly. One problem is that there are some theoretical controversies about the use of ovitrap data for assessing adult populations in high densities.[30] However, in 2009 Wan Norafikah et al. applied the ovitrap index as an indicator of a degree of infestation in specified areas.[13]

The combination of the four IVM strategies was effective in reducing the number of eggs. Furthermore, we observed a decrease in the number of eggs over time: in 2009 fewer eggs were detected in the reintervention areas compared with the intervention ones, and these differences were statistically significant, which suggests that the door-to-door communication programme can have a long-term effect on the behaviour of the population.

Collaboration of the local population is so important that 'closed' houses signify a failure of the intervention.[26] Being a key factor for success, the cooperation of the community was requested repeatedly during the house-to-house intervention. The majority of the people gave access to their property, allowing civil agents to eliminate breeding points in the gardens and back yards, and once they had been informed of the prevention measures they cooperated in the detection of potential breeding points in the areas surrounding their property.

The fact that different areas had an influence in mosquito abundance highlights the spatial heterogeneities between neighbourhoods with the same treatments but different effects owing to socioeconomic differences (e.g. characteristics of peoples' responses to the communication campaigns) and ecological differences (e.g. mosquito breeding sites, vegetation, population density). The effect of month on mosquito abundance is due to the intrinsic effect of the seasonal dynamics of the tiger mosquito, which vary over time depending on the dynamics of the meteorological variables.[31]

A greater number of containers were detected with growth of mosquito larvae and pupae in 2009 and a higher percentage of containers with standing water compared with 2008. This may be owing to the increased accessibility and inspections inside the private gardens or homes in the second year of study, and the greater involvement and cooperation of citizens.

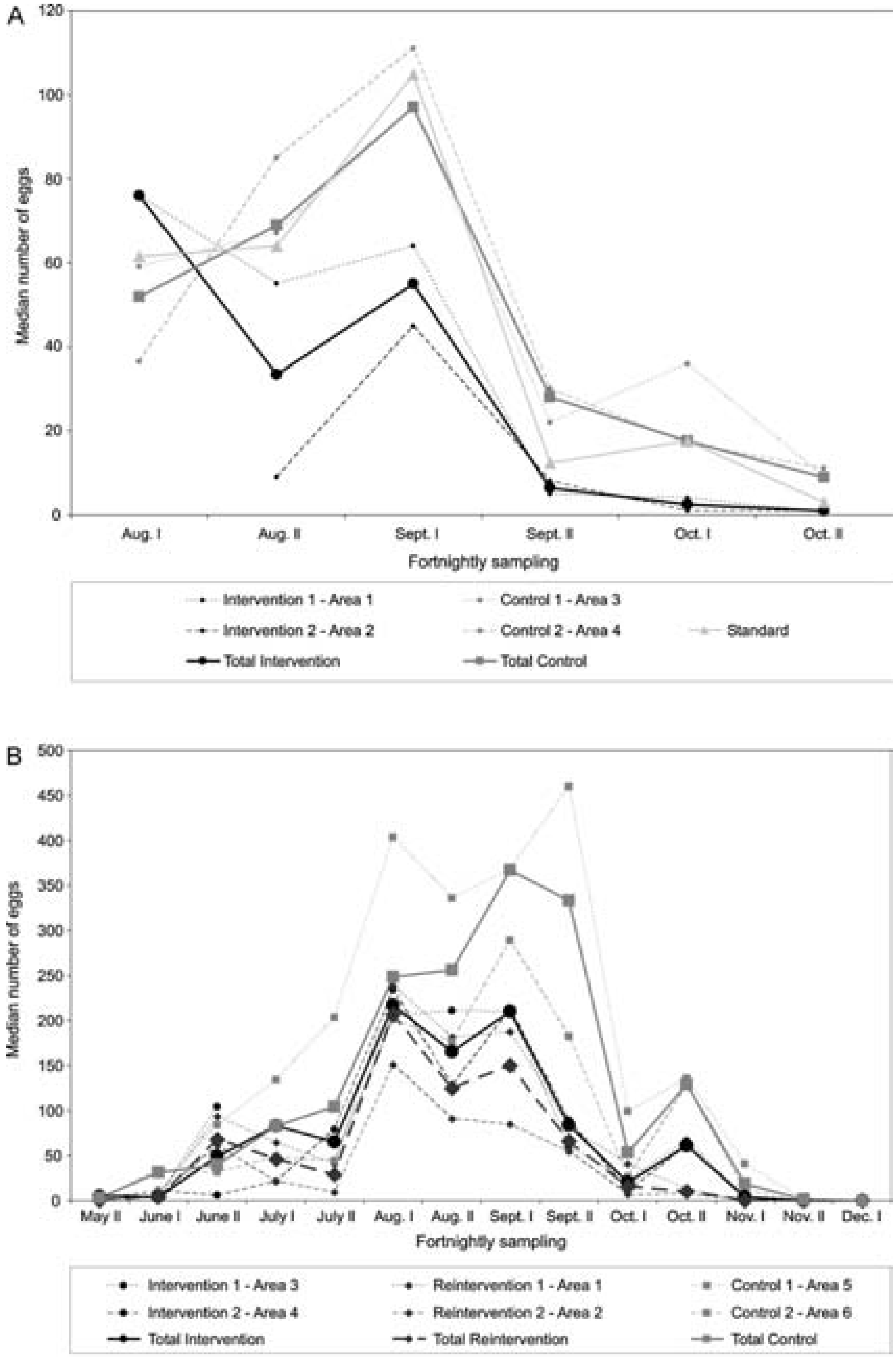

Figure 2. Evolution of the medians of eggs of *Aedes albopictus* with fortnightly sampling from August to October 2008 (A) and May to December 2009 (B).

The number of eggs increased notably in 2009 compared with 2008, showing that the mosquito population is still increasing after at least 5 years of colonization.[23] Initially, the arrival of *Ae. albopictus* was seen as very bothersome by the local people,[6] and demand for medical attention for this reason peaked in 2004,[5] diminishing thereafter. Also the cases handled by the local council diminished progressively from 2006. We consider that the reduced demand both for medical aid and help from the council was due to better knowledge of prevention and control methods and

Table 2
Results of the fortnightly sampling in 2009 using the negative binomial distribution generalized model analysis

Response variable			β (95% CI)	Std. error	Wald χ^2	df	*P*-value
Egg abundance	Intercept		2.099 (1.643–2.555)	0.2324	81.540	1	<0.001
	Factor	Control	0.721 (0.572–0.869)	0.0757	90.569	1	<0.001
		Intervention	0.155 (0.004–0.307)	0.0773	4.025	1	0.045
		Reintervention[a]					
	Covariates	Area	0.047 (0.011–0.084)	0.0186	6.445	1	0.011
		Month	0.287 (0.233–0.341)	0.0275	109.216	1	<0.001

[a] Reference category.

a possible progressive diminishing in intensity of the local immune response.

In Spain the interventions follow different local protocols, which could be improved by the application of a unified common strategy for the whole Mediterranean area. The combined four measures tested here seem to have provided benefits locally, although chemical or biological insecticidal products used may vary based on availability, regulations or evolution of resistances.[7,24] Better results of this IVM are expected, particularly if the techniques are geographically expanded and maintained for a sufficiently long period of years, and if the collaboration of the citizens is requested (enforcement laws should be considered) and education programmes permanently granted.

5. Conclusion

The combination of the four IVM strategies was clearly effective in reducing the number of eggs in the intervention areas compared with the control ones. A high level of public cooperation was obtained from the beginning, and furthermore this continued to increase as the interventions were carried out. The citizens allowed internal inspection of their properties and provided information about possible breeding points in the neighbourhood. This study could be a model for controlling the populations of *Ae. albopictus* in the Mediterranean region.

Authors' contributions: GCA conceived and designed the study, conducted the experimental field work, secured funding, extracted, analysed and interpreted the results and wrote the manuscript; DR conceived and designed the study, secured funding, analysed and interpreted the results and wrote the manuscript; RG participated in the interpretation of data and wrote the final draft; SQ and IG analysed and interpreted the results and revised the manuscript critically for intellectual content; NG conceived and designed the study, secured funding, analysed and interpreted the results and wrote the manuscript. All authors reviewed the final manuscript. GCA and NG are guarantors of the paper.

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Conflicts of interest: None declared.

Ethical approval: The verbal informed consent of the citizens was obtained. The present study was done in accordance with the Research Ethics Committee of Mútua Terrassa Hospital, Barcelona, Spain.

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Annex 2

Ajuntament de SantCugat

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QUESTIONARI **Núm. enquesta (mateix F.I) _______**

ADREÇA: ____

(Agraïm anticipadament la seva col·laboració i **garantim l'anonimat i la confidencialitat de les seves respostes)**

1 - CONEIXEMENTS SOBRE MOSQUIT TIGRE I PREVENCIÓ

1.- Havia sentit vostè parlar del mosquit tigre prèviament a aquesta visita?
□Si □No □NS/NC
2.- Disposa vostè d'informació sobre la prevenció del mosquit tigre? □Si □No
3.- Ha rebut vostè informació sobre el mosquit tigre? A través de: (Resposta múltiple)
□ Premsa, radio, TV □ Internet
□ Fullets informatius □ Boca a boca
□ Personal sanitari □ Ajuntament
□ Informadores
□ Altres. ¿Quin?________________________________
□ No
4.- Considera vostè necessari que se l'informi més sobre el mosquit tigre? □Si □No
Comentaris:____
5.- Coneix vostè on es reprodueix el mosquit tigre? □Si □No
6.- Pren vostè alguna mesura preventiva contra el mosquit tigre i/o les seves picades? (Resposta múltiple)
□ Repel·lents
□ Fumigar
□ Barreres físiques (mosquitera i altres)
□ Evitar estancament d'aigua
□ Altres Quines?________________________________
□ Cap
Comentaris____
____

7.- Pateix vostè algun problema relacionat amb el mosquit tigre ara? □Si □No
8.- Va patir vostè aquest problema als darrers anys? □Si □No
9.- En la seva experiència, quin va ser l'any "pitjor" respecte a les picades?
□2002 □2003 □2004 □2005 □2006 □2007 □2008 □2009 □2010 □2011 □2012
10.-Ha vist alguna vegada al mosquit tigre? □Si □No

2 – QUALITAT DE VIDA i DADES CLÍNIQUES:

11.-Considera vostè un problema el mosquit tigre? □Si □No

12.-Dels següents altres insectes, hi ha algun d'ells que consideri vostè un problema?
□ Mosquits comuns
□ Mosca negra
□ Abelles
□ Vespes
□ Cap insecte és un problema per a mi.
□ Altres_______________________________________

13.- Vostè creu que les picades d'insecte han afectat al seu ritme de vida habitual o de la seva família?
□Si □No □NS/NC

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14.- Com? En quins aspectes:
□ Utilització del jardí.
□ Utilització de la piscina.
□ Canvis en la forma de vestir.
□ Canvis en la llar (mosquiteres i altres).
□ Canvis en la vida social (invitats que no venen i altres).
□ Altres. Especificar____
15.- Nombre de persones afectades a la vivenda ______
16.- Nombre de persones a la vivenda ______________
17.- Té vostè la impressió que el mosquit tigre té preferència per algú dels habitants de la vivenda?
□Sí □No □Ho desconeix
18.- Per quina persona té preferència? Edat ________ Sexe: □Home □Dona
19.- A que ho atribueix?____
20.- Si us plau pensi en la darrera picada (la més recent) que va sofrir: Quin Insecte : _______________
a) Recorda vostè el nombre de picades:_______
b) Recorda la localització de la picada : □Cames □Braços □Tronc □ Cap
Concretament____
c) Recorda el tipus de lesió/ns que li va provocar la/s picada/es:
□ Envermelliment □ Fava (enduriment) □ Butllofa (líquid) □ Hemorràgia □ Altres_____________
d) Recorda les molèsties provocades per les picades:
□ Picor lleuger □ Picor moderat □ Picor intens □ Dolor
e) Se li va infectar a vostè la picada? □Si □No □Ho desconeix
On va ser vostè atès al patir la picada? (respostes múltiples)
□ CAP □ Urgències hospitalàries □ Metge privat □ Farmàcia□ No va consultar
□ Altres __________
f) Li van prescriure algun tipus de tractament?
□Si. Quin? ____
□No
□No se'n recorda

g) Es va autoadministrar vostè, pel seu compte, algun tipus de tractament?
□Si. Quin? ____
□No
Comentaris:____
____
21.- Si us plau pensi ara en els canvis que vostè ha percebut en les picades rebudes durant els darrers 7 anys
a) Ha rebut al menys una picada d'insectes al any, vostè o els seus familiars o persones més propers?
□Sí □No □No se'n recorda
b) Té la percepció de que durant aquests anys aquestes picades rebudes li causen:
□ molèsties similars cada any
□ una reacció que tendeix a disminuir (els primers anys o les primeres vegades van ser més intensa).
□ una reacció que tendeix a empitjorar (els primers anys o les primeres vegades van ser més lleugeres)

3 – ALTRES DADES DELS VOLTANTS
21.- Coneix algun punt de cria de mosquits en el seu entorn o al barri? □Si □No
Especificar____
22.- Ha percebut canvis respecte al mosquit tigre en el seu barri els darrers anys?
□Sí □No □No se'n recorda
23.- Quins canvis ha percebut _______________________________________ _________ ___________
____
24.- En la seva opinió, qui creu que ha de solucionar el problema?
Especificar____

4 - DADES SOCIODEMOGRÀFIQUES
a) Edat de la persona entrevistada______
b) Sexe: □Home □Dona
c) Escolarització de la persona entrevistada □ Sense estudis □ Bàsica □ Mitja (FP, batxillerat) □ Superior

Moltes gràcies per la seva col·laboració!

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FULL D'INSPECCIÓ Número full____________________

DATA__________BARRI__________________SECTOR____________________

ADREÇA____

DADES SOCIODEMOGRÀFIQUES (Només quan es realitza una entrevista)

a) Edat (categoritzada, només si no s'ha respost a la enquesta). ________
b) □Home □Dona Càrrec familiar_________________
c) Nivell socioeconòmic: □baix □mig □alt

1 –CONEIXEMENTS (Del punt de vist de l'informador/a)

Coneix vostè on es reprodueix el mosquit tigre? □Realment era conscient de la reproducció en dipòsits petits d'aigua estancada.

2 - TIPUS DE VISITA 1- □ Periòdica (especificar)______________________

2- □ Puntual (2a o 2b): 2-a □ Porta a porta 2- b □ Denúncia

3- HABITATGE

□ **DES DE L'EXTERIOR (pregunta 1)** □ **DES DE L'INTERIOR (pregunta 2)**

1 Des de l'exterior es veu: □ Res □ Poc □ Bastant □ Molt

2 - Tipus d'habitatge: □Comunitat □Primer domicili □Segon domicili □Comerç □Terreny □Deshabitada □Ruïna □Comerç □Escola □Obres

3 - Zona exterior de l'habitatge: □Patí (cimentat) □Jardí □Terreny boscós □Zona comunitària □Terrassa □Piscina tractada □Hort □Galliner

4- INSPECCIÓ (DADES AMBIENTALS)

1 - Els recipients poden acumular aigua? □ Sí □ No □ No se sap

2 - Quants?______

3 - Tipus de focus possible: □Piscina □Bassa □Dipòsit □Bidons □Galledes □Font □Safareig □Neumàtic □Embornal □Ampolles □Plats de testos □Joguines □Deixalles □Altres

Especificar____

4 - Hi ha actualment cap focus actiu? □Sí □No □No se sap.

5 - Quants?______

6 - Tipus focus actius: □Piscina □Bassa □Dipòsit □Bidons □Galleda □Font □Safareig □Neumàtic □Embornal □Ampolles □Plats de testos □Joguines □Deixalles □Altres

Especificar____

7 - Dimensions (Àrea/volum)____ __

8 - Focus tractat (per l'agent cívic) al moment de la inspecció? □ Sí □ No

9 - Tipus de tractament □*Bti* □Diflubenzuron □Buidat

10 - Mostra larves? □SÍ □No 11- Quina espècie? □ Culex □ Aedes □ Altres__________

12 - Picades? □ Si □ No 13- Quantes?_______

14 - Presència de mosquits en fase aèria? □ Si □ No

15 - Factors de risc: □ Vegetació densa □Material obra □Restes de poda □Llenya □Residus □ Altres

Especificar____

16 - Nivell de risc □Cap □Baix □Mig □Alt □No es pot concretar

17 - Avís a la porta? □ Si □ No

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5 - ENTORN

18 - Entorn de l'habitatge (100 m): □Riera □Zona Verda/terreny boscós □Hab. deshabitadA □Hab. amb focus actius □Hab. amb focus potencials □Punt amb vegetació densa □Obres □Abocaments incontrolats □Altres (especificar) ________________________________ _

19 -Punt/s amb aigua estancada a la via pública? □Sí □ No □ No se sap

20 -Quin tipus?________________________________ 21 -Quants? ___________________

22- Punt/s amb focus actius? □ Si □ No □ No se sap 23-Quin tipus?__________________________________

24- Quants? ____________Dimensions____

25- Focus tractat al moment de la inspecció? □Sí □No

26-Tipus de tractament □*Bti* □Diflubenzuron □Buidat

Observacions____

____

____

MESURES PREVENTIVES CONTRA EL MOSQUIT TIGRE

Ajuntament de SantCugat

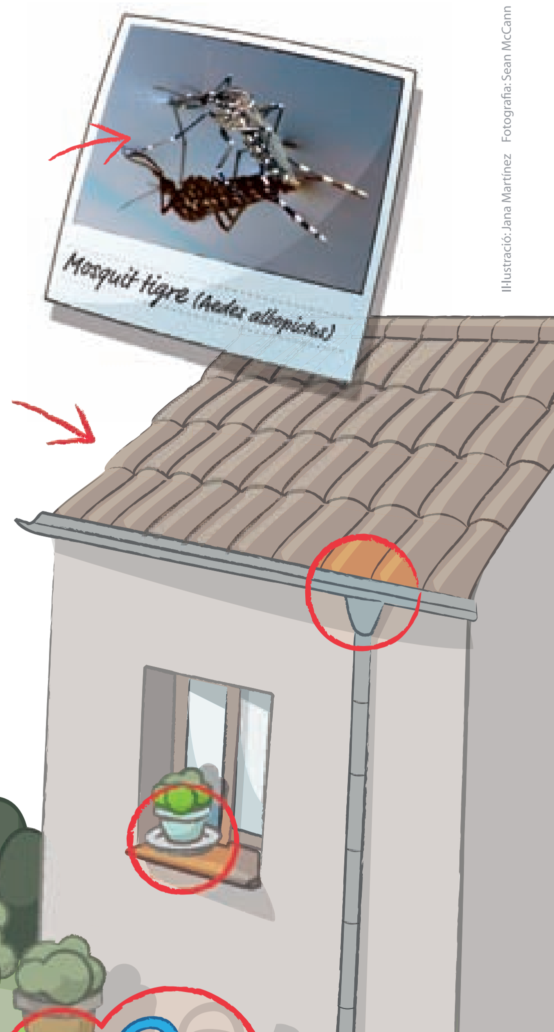
El mosquit tigre és un insecte invasor que actualment està instal·lat al nostre municipi. El cicle biològic del mosquit tigre inclou una fase aquàtica (larves) i una fase aèria (adulta).

La femella diposita els ous en espais susceptibles de ser inundats i és en l'aigua estancada on es desenvolupen les larves. És actuant en la fase aquàtica quan **la vostra col·laboració és imprescindible per evitar que les larves es desenvolupin** fins a la fase adulta de l'insecte.

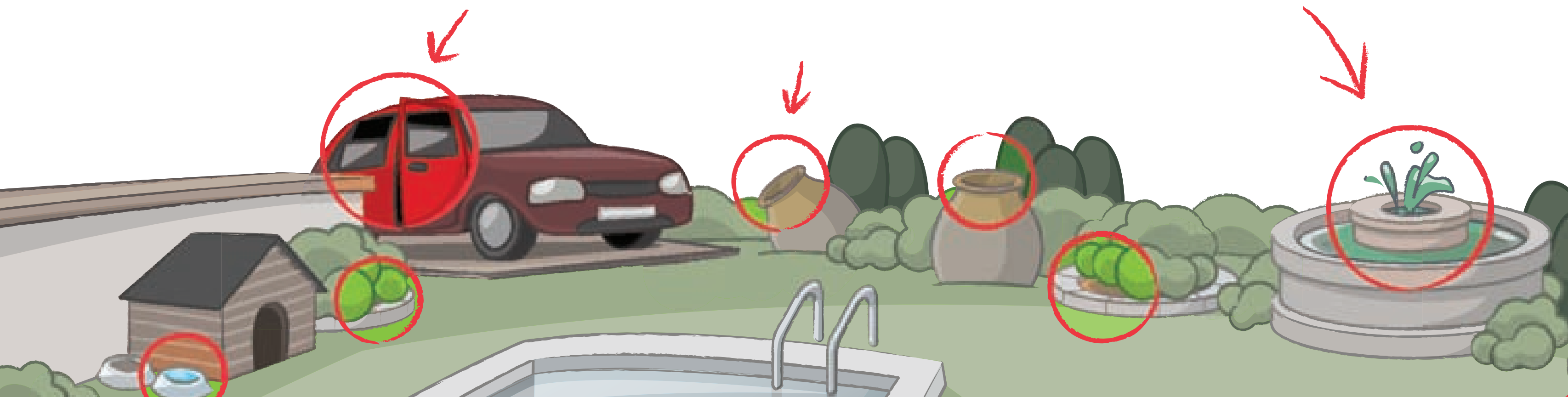
Si apliqueu les següents mesures preventives, entre tots reduirem les molèsties que provoca la presència dels mosquits.

- Tractar l'aigua de les piscines durant tot l'any o tapar-les hermèticament.
- Evitar tenir aigua entollada sense tractar: basses, abeuradors, piscines inflables, estanyols o fonts. Si no es poden eliminar podeu posar-hi peixos (Carpí vermell) que s'alimenten de les larves.
- Controlar que no s'acumuli aigua als desguassos o als canelons de la teulada.
- Eliminar els recipients de l'exterior que puguin acumular aigua: gerros, testos, plats, pots, joguines, regadores, ornaments, ...
- Cobrir els espais on s'acumuli aigua que no puguin ser eliminats, amb tela mosquitera o omplir-los de sorra.
- Canviar diàriament l'aigua dels abeuradors dels animals domèstics i dos cops per setmana l'aigua de les plantes aquàtiques.
- Tancar les portes i les finestres dels vehicles estacionats, per tal d'evitar el transport passiu de l'insecte.
- Mantenir la vegetació controlada i retirar els objectes, deixalles o materials en desús per evitar que serveixen d'amagatall de mosquits.

Per a més informació, truqueu al telèfon **010**, o consulteu la pàgina web de l'Ajuntament (www.santcugat.cat)



Il·lustració: Jana Martínez   Fotografia: Sean McCann



Annex 3

Ajuntament de SantCugat

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PLA DE TREBALL

Campanya de prevenció i control del mosquit tigre 2012

La campanya de 2012 proposa treballar seguint criteris de control integrat: accions d'informació, difusió i participació i accions de vigilància i control de l'expansió.

Les intervencions de la campanya segons els mesos de gener a desembre estan reunits en la Taula 1

1. ACCIONS D'INFORMACIÓ, DIFUSIÓ I PARTICIPACIÓ

Objectius:

L'objectiu de la campanya informativa és conscienciar la població sobre les mesures de prevenció per evitar la presència del mosquit tigre a la llar. Més allà de l'eliminació dels punts de cria de mosquits, les persones que viuen a les zones afectades són animades a participar activament de la campanya al seu barri o zona. Les formes de participació poden ser en diferents àmbits, sigui en permetre la inspecció al seu habitatge o en indicar possibles punts de cria al barri, entre altres

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Taula1 – Calendari d’actuacions – campanya prevenció i control del mosquit tigre (*Aedes albopictus*) en Sant Cugat del vallès.

Accions / Mesos	1 2 12	3 4	5	6 7	8	9 10	11
Secció educativa de la campanya – Participació ciutadana							
Campanya porta a porta			🟩	🟩	🟩	🟩	
Educació ambiental (pla de dinamització educativa)			🟩	🟩		🟩	🟩
Conferències i tallers a centres socials, casals i altres.		▒	🟩	🟩	▒	🟩	🟩
Comunicació: Web municipal, premsa local, cartes a empreses de risc i campanya al cementiri.		🟩	🟩	🟩	🟩	🟩	
Formació de personal municipal			🟩	🟩			
Secció Vigilància ambiental i control del mosquit tigre							
Atenció al ciutadà	▒	🟩	🟩	🟩	🟩	🟩	🟩
Seguiment d’expedients i incidències	▒	🟩	🟩	🟩	🟩	🟩	🟩
Instal·lació i monitoratge de paranys BGS (adults) i d’oviposició		🟩	🟩	🟩	🟩	🟩	🟩
Inspeccions i tractaments larvicides embornals (via pública i privada)		🟩	🟩	🟩	🟩	🟩	🟩
Tractaments insecticides*				🟨	🟨	🟨	

*Realitzats per empresa especialitzada quan cal.

🟩 Intervenció obligatòria

▒ Baixa incidència d’entrada de reclamacions (hivern/vacances)

🟨 Intervenció optativa, segons resultats del monitoratge amb els paranys

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1.1. Campanya porta a porta

La població afectada té un paper molt important en el control del mosquit tigre, la seva contribució es concentra en l'eliminació dels punts de cria a les seves llars. La campanya porta a porta és una intervenció eficaç per informar i assessorar *in situ* la població sobre l'eliminació de l'aigua estancada durant la temporada de mosquits, en general de maig a octubre.

Les visites es realitzaran a les zones que en les campanyes anteriors han concentrat les denúncies i reclamacions del veïnat. Els districtes de Mira-sol, Valldoreix i La Floresta seran prioritzades, seguides de Les Planes i Can Barata. Pel que fa el Nucli urbà, les accions es concentraran a Coll Favà, Sant Domènec, Casc Antic, Can Trabal i Eixample Sud.

- **Campanya informativa porta a porta** (condicionada a la incorporació de 4 treballadors/es a través d'un pla d'ocupació)**:**

  Després de rebre la formació necessària (aproximadament 3 setmanes), l'equip treballarà en l'execució de la campanya de sensibilització ciutadana. Previsió 1500-2000 visites "porta-a-porta" per temporada.

- **Campanya porta a porta (seguiment expedients):** Visites "porta a porta" als habitatges de on provenen denúncies i a altres propers al domicili. Previsió 250-400 visites per temporada.

  S'entrevista una persona adulta que habita al domicili seguint un protocol de valoració del nivell de coneixement sobre prevenció i de la presència de focus de cria de mosquit tigre. Els habitatges són classificats segons el seu nivell de risc i són georeferenciat amb un dispositiu GPS. Els resultats de les inspeccions estaran publicades a INTRANET, on es podrà visualitzar a través del GIS tots els habitatges inspeccionats (http://www.gis.santcugat.cat/mosquit) (Figura 1)

  A cada visita es lliurarà un full il·lustrat amb les mesures preventives

1.2. Intervenció en l'àmbit educatiu

Taller pràctic dirigit a alumnes entre 9 i 13 anys (5è-6è de primària i 1r-2n d'ESO) Té com a objectiu reforçar les accions que els/les estudiants puguin realitzar a casa o al seu voltant i que afecten directament la densitat de mosquits a la llar.

La metodologia del taller es basa en l'educació ambiental (EA) amb el repte de canviar el comportament i promoure la participació activa dels/ de les ciutadans/es en base al coneixement i l'experiència.

1.3 . Conferències i tallers a centres socials, casals i altres

Oferta de conferències informatives amb l'objectiu de promoure el coneixement sobre les mesures preventives a les poblacions afectades. El cartell informativo utilitzado en la divulgació de la conferència està disposat al final d'aquest document.

Exposició oral seguida d'un taller pràctic i taula rodona, on els/les ciutadans/es tenen l'oportunitat de conèixer el cicle biològic de l'espècie (ous, larves, pupes i mosquits adults), d'identificar els punts de cria i de participar en la planificació de la campanya al

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seu barri. Els locals idonis per a la realització d'aquestes conferències són els centre cívics i casals dels districtes (Realització: Gisela Chebabi. Durada 1-2 hores)

1.4. Comunicació

La campanya de comunicació inclou:

- **Informació general a la premsa local i el web municipal**: l'eix de la informació proporcionada serà:: "Què puc fer per evitar la presència de mosquits a casa?"; "Tinc mosquits a casa, què puc fer per baixar la seva densitat?"i "Com evitar i curar les picades?"
- **Informació a empreses d'alt risc** de posseir punts de cria de mosquit. Concretament empreses de construcció, manipulació de pneumàtics (compra, venda i reparacions), botigues de jardineria i hípiques.
- **Difusió de material informatiu** a tots els edificis municipals, a farmàcies i CAPs . Material disponible d'altres campanyes (cartells i tríptics distribuïts per l'Agència de Protecció de la Salut de la Generalitat de Catalunya)
- **Campanya cementiri**: intervenció específica dirigida a les persones que posen gerros amb aigua estancada. L'objectiu és conscienciar la població sobre el risc d'infestació de mosquits al cementiri per tal d'evitar els tractaments insecticides amb la nebulització de piretroides. El model de cartell que s'utilitzarà a la campanya està disposat al final del document.

1.5. Formació de personal municipal (Policia, OAC, S. Socials i Brigada)

Taller pràctic de formació per al personal municipal que està més en contacte amb la ciutadania i/o treballa a la via pública. L'objectiu és dotar-los dels coneixements que els permetin assessorar la població afectada i detectar punts de creixement larval als habitatges i a la via pública. (Realització: Gisela Chebabi. Durada 1 hora)

2. ACCIONS DE VIGILÀNCIA AMBIENTAL I CONTROL DEL MOSQUIT TIGRE

Objectius:

L'objectiu del control del mosquit tigre és reduir la seva població per sota d'un llindar de tolerància. Això significa disminuir l'abundància de l'espècie fins a que no representi una molèstia, tot i que segueixi present. La vigilància ambiental es constitueix del monitoratge de l'activitat i la dinàmica del insecte a través de diferents eines (trampes, reclamacions, etc). D'aquesta manera es pot conèixer i prioritzar els punts de més abundància de mosquits i facilitar el seu control al municipi.

2.1. Atenció ciutadana

Atenció a les trucades sobre mosquits, proporcionant informació bàsica i programant les inspeccions *in situ*.

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2.2. Incidències i seguiment d'expedients

Les incidències habitualment són denúncies referents a focus de cria o a alta densitat de mosquits.

Cada incidència determinarà l'obertura d'un expedient, inspecció i seguiment.amb localització geogràfica i informes tècnics fins al tancament.

2.3. Instal·lació i monitoratge de paranys BG-Sentinel (adults)

Es comptarà amb 10 paranys de mosquits adults i 10 paranys d'oviposició que seran instal·lats de juny a octubre a les escoles amb més freqüència d'incidències per mosquits:

- Escola Bressol El Niu
- Escola Bressol El Gargot
- Escola Bressol Cavall Fort
- Escola Bressol El Tricicle
- Escola Pins del Vallès
- Escola Ferran i Clua
- Escola Catalunya
- Escola Pi d'en Xandri
- Escola Turó de Can Mates

Els paranys seran supervisats quinzenalment, es faran recomptes del nombre de mosquits capturats i del nombre d'ous a les fustes de les ovitrampes. L'objectiu d'aquest monitoratge és estimar l'abundància de la població de mosquits tigre per poder prioritzar les zones l'alta incidència i realitzar mesures correctores.

2.4. Inspeccions i tractaments larvicides.

A partir del moment climatològic en que les temperatures mínimes diàries arriben als 12ºC s'inicia l'època en que el mosquit tigre es fa present. A partir d'aquell moment s'iniciarà el control periòdic dels punts permanents de cria..

Els edificis municipals, places, parcs públics i carrers que tenen embornals o fonts amb aigua permanentment estancada estan detallats en la figura 1, que espot accedir aquí. A Es portarà a terme tractament larvicida (larvicides biorracionals biològics com el Vectobac (*Bacillus thuringiensis israelensis* – bti) i químics com el diflubenzuron)

2.5 – Tractaments insecticides

L'ús racional d'insecticides ha demostrat ser útil per eliminar un gran número de mosquits adults, incrementant l'eficàcia del control integrat. Tanmateix, l'aplicació d'adulticides es restringirà a situacions d'alt risc.

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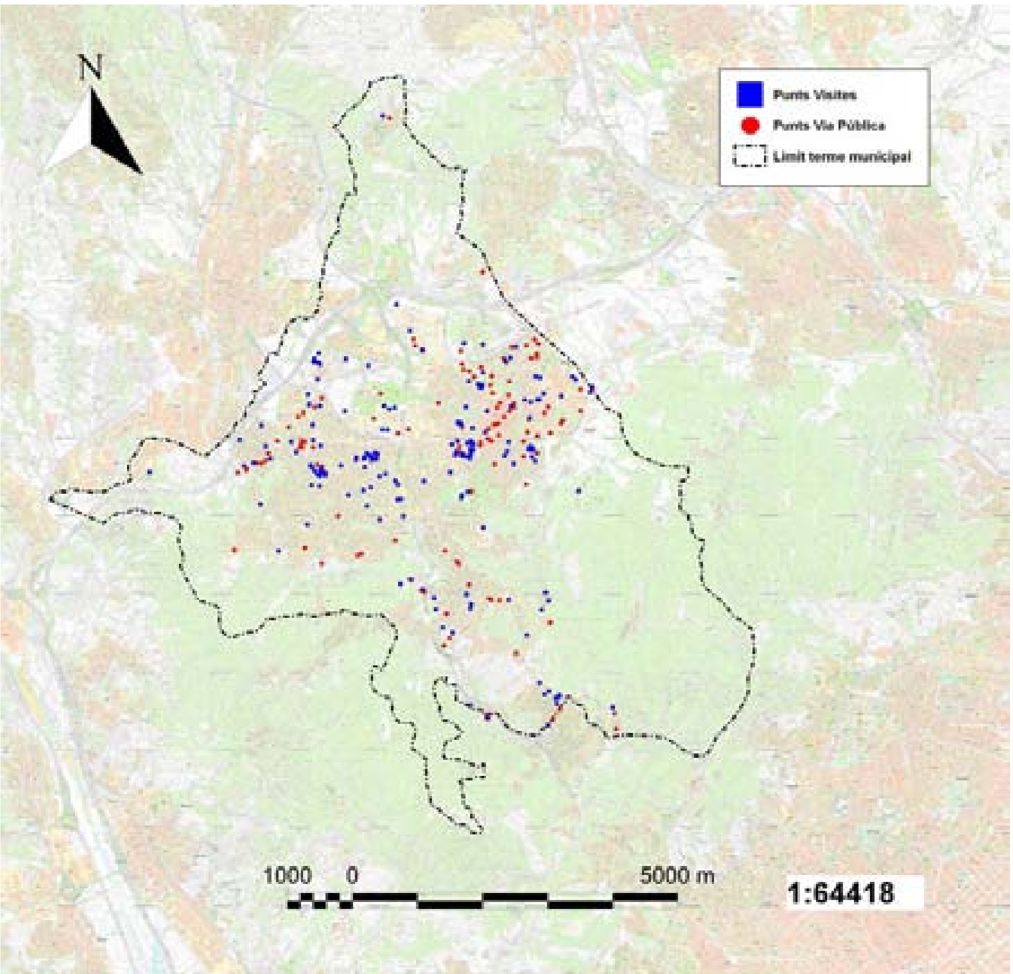
GIS

Els punts de risc que cal inspeccionar i controlar periòdicament , referents a la via pública (embornals, fonts), rieres, parcs i jardins, equipaments municipals i propietats privades seran georeferenciats amb un dispositiu GPS.

La visualització de gran part dels punts calents situats en la via pública estan publicades a l'WEB municipal: http://www.gis.santcugat.cat/mosquit

En aquesta mateixa pàgina també es podrà consultar l'estudi realitzat el municipi (2008-2009) on es van dur a terme una estratègia de control integrat amb l'aplicació d'intervencions combinades per reduir la densitat de mosquits al municipi.

Figura 1 – Visites puntuals i preriòdiques realitzades a Sant Cugat – campanya 2011



Font: Web municipal Sant Cugat http://www.gis.santcugat.cat/mosquit

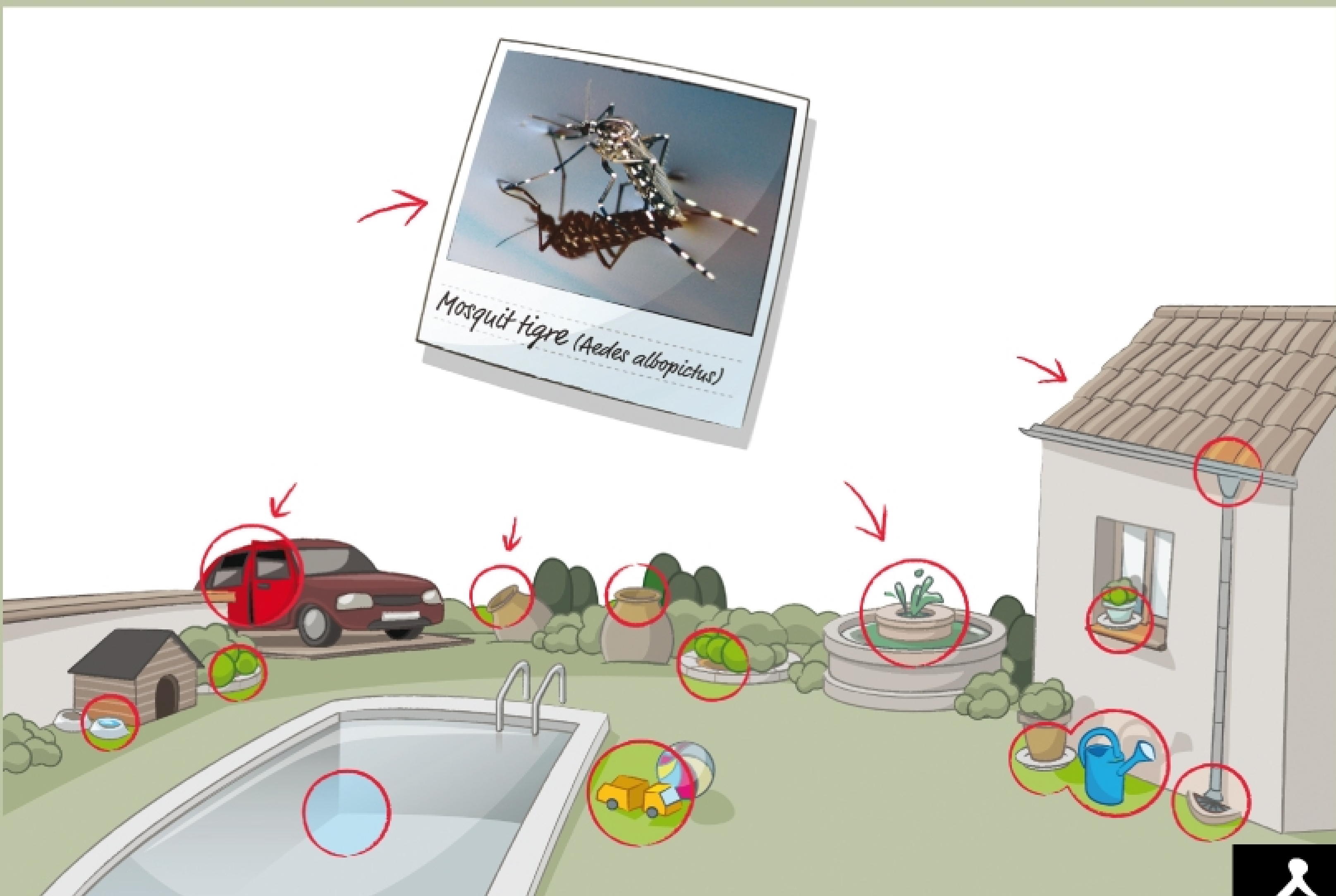
(Última consulta setembre 2012)

xerrada

El mosquit tigre

La participació ciutadana com a eina de control

13 de juny
19 h
Casal Cultural de Mira-sol



Ajuntament de SantCugat

PREVENCIÓ CONTRA EL MOSQUIT TIGRE

no donis vol al mosquit tigre

→ Per conservar les flors utilitza boletes d'hidrogel o sorra humida, tindran la mateixa durada i evitarem la seva proliferació

Ajuntament de SantCugat