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E

MICROBIOLOGIA

AEDES ALBOPICTUS ADULT DISPERSAL

IN DIFFERENT ECOLOGICAL CONTEXTS IN ITALY

CANDIDATO

Francesca MARINI

DOCENTE GUIDA

Dr. Alessandra DELLA TORRE

COORDINATORE

Prof. Gianfranco TARSITANI

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ABSTRACT

Le elevate densità raggiunte da *Aedes albopictus* in molte città italiane rappresentano non solo un problema ecto-parassitario, ma anche un rischio di trasmissione di arbovirus, soprattutto in relazione alla possibile importazione di arbovirus patogeni (es. Dengue e Febbre Gialla) da parte di viaggiatori o migranti provenienti da aree endemiche. In effetti, nell'agosto del 2007 nella provincia di Ravenna, *Ae. albopictus* è stata responsabile di una epidemia del virus Chikungunya che ha causato più di 200 casi di malattia confermati, a partire da un singolo individuo infetto recentemente arrivato in Italia dall'India. L'ampia diffusione di questo potenziale vettore in Italia impone una maggiore conoscenza di alcuni aspetti della sua biologia utili alla valutazione del rischio di trasmissione di patogeni ed alla corretta pianificazione delle azioni di controllo, quali le abitudini di puntura e di riposo e la capacità di dispersione in funzione della ricerca dell'ospite oppure dei siti di ovideposizione.

L'obiettivo della presente tesi di dottorato è l'analisi della capacità di dispersione di *Ae. albopictus* in differenti contesti ecologici italiani. Infatti, la maggior parte delle conoscenze oggi a disposizione su questo ed altri aspetti della biologia di *Ae. albopictus* derivano da studi effettuati in regioni del sud-est asiatico e del continente sud-americano. Tali informazioni, riferendosi a contesti molto differenti da quelli delle aree temperate, sono difficilmente applicabili alle popolazioni di *Ae. albopictus* del nostro Paese.

Sono stati condotti esperimenti di Marcaggio-Rilascio-Ricattura in un'area di 250 m di raggio all'interno della Città Universitaria, dell'Università "Sapienza" di Roma (RM) nel 2008, ed in un'area rurale/periurbana, di 500 m di raggio nel Comune di Piove di Sacco, in provincia di Padova (PD) nel 2009. Ogni esperimento è stato replicato 3 volte rilasciando circa 500 femmine *blood-fed* a RM e circa 1.000 a PD. La ricattura degli individui rilasciati è stata effettuata mediante l'uso di 55 *sticky traps* a RM, e 96 a PD, per un periodo di circa 16 giorni dopo il rilascio.

I tassi di ricattura ottenuti variano tra 3,3–5,1% a RM e 3,4–13,4% a PD. La maggior parte delle femmine sono state ricatturate gravide e nei primi 8 giorni dal rilascio. Questa osservazione, in accordo anche con i risultati di esperimenti di deposizione eseguiti in contemporanea, consente di concludere che quanto osservato si riferisce principalmente alla dispersione delle femmine in cerca di un sito di ovideposizione, al termine del ciclo gonotrofico attivato dal pasto sangue offerto loro prima del rilascio. Le femmine sono state ricatturate prevalentemente a 50-200 metri dal sito rilascio a RM e 0-150 m a PD. La distanza media percorsa è stata 105 - 139 m nell'area di RM e 68 – 110 m in quella di PD. In entrambi i siti le femmine hanno dimostrato di essere in grado di raggiungere il limite dell'area di studio (distanza massima osservata = 230 m a RM, 464 m a PD).

Nel complesso, questi risultati consentono una prima valutazione delle capacità di dispersione delle femmine di *Ae. albopictus* in Europa e dimostrano l'abilità di questa specie di spostarsi per centinaia di metri in pochi giorni, anche in condizioni di ampia disponibilità di siti di ovideposizione. E' pertanto possibile ipotizzare che le distanze coperte potrebbero aumentare sotto forti pressioni legate, ad esempio, alla scarsità di siti di ovideposizione o di ospiti su cui compiere il pasto di sangue. Questa conclusione è particolarmente importante nella pianificazione di attività di controllo in Italia, così come in altri Paesi europei, soprattutto qualora si miri ad interrompere la trasmissione di agenti patogeni nel corso di epidemie di arbovirus.

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1.INTRODUCTION

1.1. GEOGRAPHICAL DISTRIBUTION

Aedes albopictus, commonly referred to as the "tiger mosquito", origins from south-east Asia and occurs throughout the Oriental Region from the tropics of Southeast Asia, the Pacific and Indian Ocean Islands, north through China and Japan and west to Madagascar. Over the last three decades this species has spread from the western Pacific and South-east Asia to Europe, Africa, the Middle East, North and South America and the Caribbean (Figure 1).
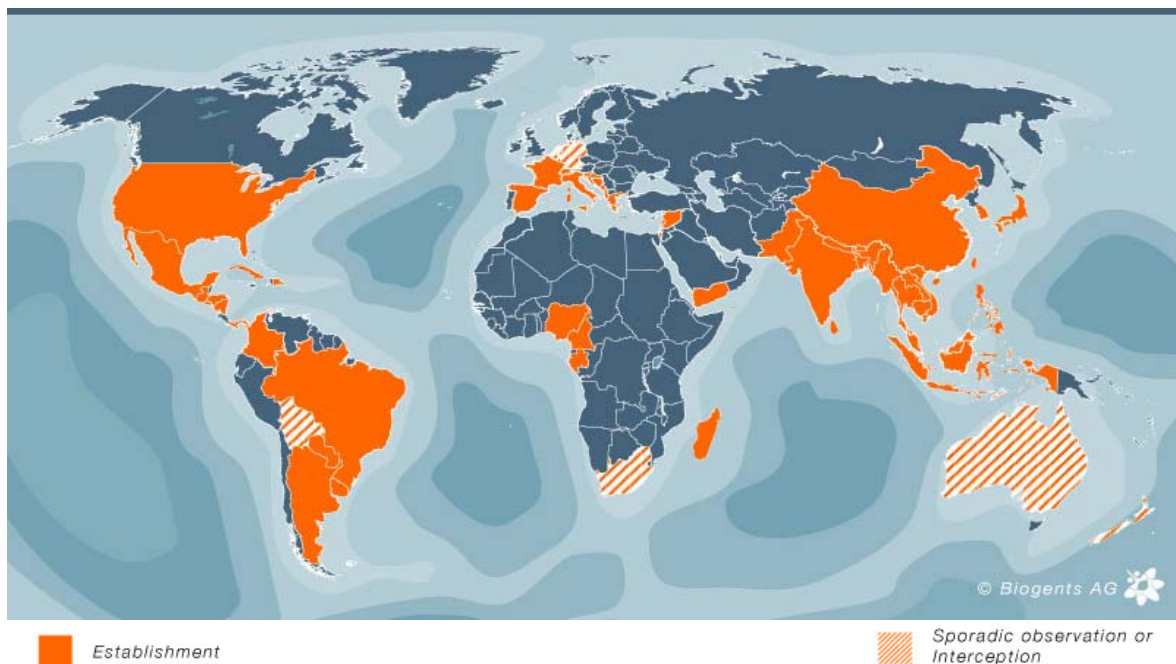

Figure 1 – Global distribution of *Aedes albopictus*, 2008.

The main means of dispersion of *Ae. albopictus* are as dormant eggs in used tires. In fact, the trade of used tires plays a important role in the global economy, because they can be used in several ways, such as recapping/repair, fuel, artificial reefs, crash barriers, soil erosion control, asphalt, sandals, gaskets, outdoor playground surfaces, and running tracks. Usually these tires are stored outside, where they can collect rainwater and organic matter (e.g. dead leaves), providing breeding sites for some species of *Culicidae. Ae. albopictus* has showed a easy adaptability to several artificial breeding sites, among which the tires. Eggs are laid on the inside surface of the tire and are carried across the world trough used-tires commercial exchanges [1].

Aedes albopictus has found for the first time in Europe in 1979 in Albania [2], then has been reported in Italy [3], France [4], Serbia and Montenegro [5], Belgium [6], Switzerland [7], Greece [8] and Spain [9] (Figure 2).
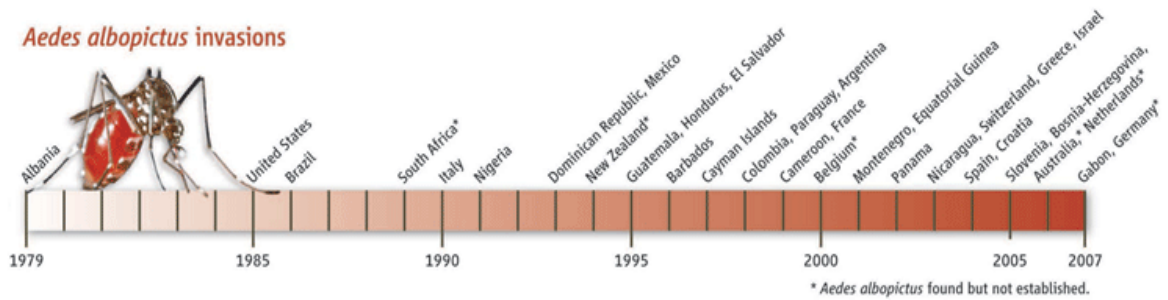
Figure 2 - Temporal trend of invasions of *Aedes albopictus* in the world [79].

Today *Ae. albopictus* is the most serious pest mosquito species in Italy [10]. This specie was found for the first time in Genoa in September 1990 as a few adults of unknown origin [3]. An established population was found 1 year later near Padua [11]. This species rapidly spread across the northern and central regions of Italy by trading of used tire, up to reaching Rome in 1997. Now it is present in the great part of the Country (Figure 3). A study has showed that the infestation has originated in a tire depot that received eggs infested shipments of aircraft tires from Atlanta, U.S. Further genetic
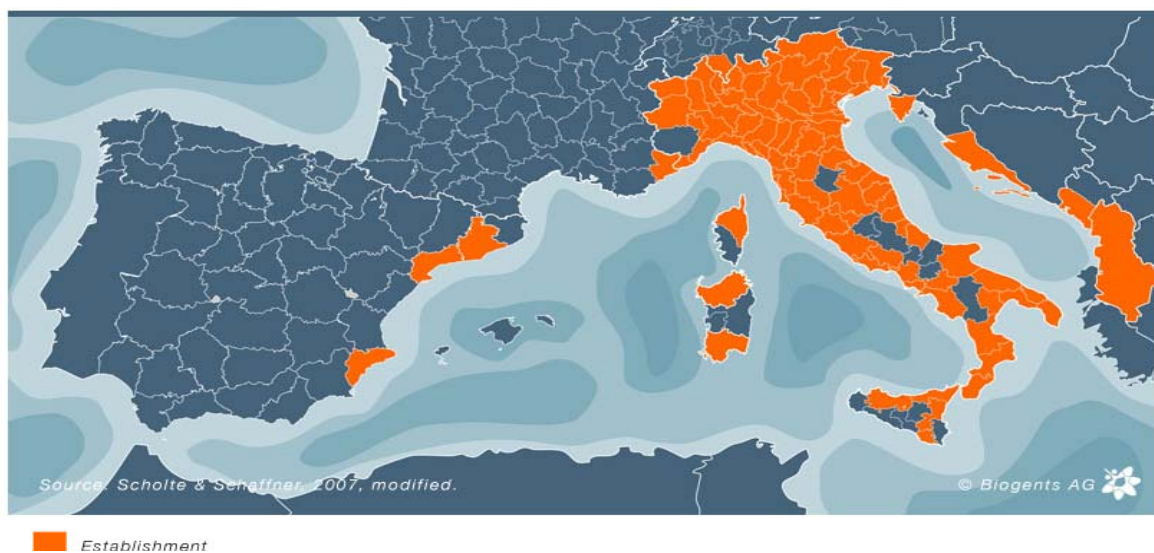
Figure 3 – European and Italian distribution of *Aedes albopictus*, 2007.

analysis showed affinity between Italian, U.S. and Japanese *Ae. albopictus* [12]. Because of its quickly spread, it is timely to assess the extent to which this specie is or may be a disease vector, particularly in those areas into which it has been introduced. This information is very important to public health and for management and control of this specie [13].

1.2.   GENERAL BIOLOGY OF *AEDES ALBOPICTUS*

Mosquitoes have four distinct stages in their life cycle, namely, egg, larva, pupa and adult. The first three stages are passed in water but the adult is an active, flying insect that feeds on blood or plant juices.

`Egg biology` – The eggs of *Aedes* mosquitoes have not a frill or floats but elongate-oval in shape. The outer shells of the eggs are patterned with small reticulations. The eggs are white in color and soft when they were newly laid, but later the eggs turn black and quite hard. The gravid mosquito females lay eggs above the water surface or on the damp substrates. The eggs are normally laid above the water line of breeding places. Eggs of *Aedes* mosquito may require repeated immersions in water followed by short periods of desiccation before to hatch [14]. Moreover the eggs of *Ae. albopictus* are able to resist for several months to desiccation allowing to this species to survive during dry season in tropical areas. Some Mosquito Tiger populations have diapausing eggs that are able to resist to temperatures below zero degrees during winter and hatch the following spring when temperatures and photoperiod became suitable. This characteristic gives to this species the possibility to colonize temperate regions surviving during the cold winter months. Eggs hatching is influenced by a complex interplay of factors (age, desiccation, temperature changes, oxygen tension and photoperiod). How all these factors determine whether or not an *Ae. albopictus* egg hatches upon flooding it has yet to be clarified.

`Larval biology` – Mosquito larvae undergo four larval stages that require five to 10 days for completion. Low temperatures slow the development rate [15]. Increased larval density or decreased food supply cause an increase of larval mortality and decrease in adult size [16]. Mosquito larvae are never found in turbulent waters because the larva unable to withstand wave action. The larvae commonly are found in waters containing microflora and fauna and debris of plant and animal origin). Mosquito larvae move about mainly in two ways; by jerks of the body and by propulsion with the mouth brushes. Mosquito larvae normally dive to the bottom when the water surface is suddenly disturbed or if a shadow passes over them. Mosquito have four larval instars. The first instar is recognized by the presence of an egg-breaker on the dorsum of the head and consists of a strongly sclerotised, very sharp, pointed, curved and flattened cone set in an oval area of soft membrane. It is used to cut through the egg shell and allow the larva to escape by simply forcing off a circular cup from the anterior end of the egg. The second instar larva, is much the same in length as the fully grown first instar, but is bulkier and the swollen head is enormous. As in the first instar, the head darkens and

the body becomes long and cylindrical. During this instar the larva grows in length from 2 to 3 mm. The third instar larva has certain characters of its own and the head measurement is more variable than in any other stage, since there are large and small headed forms. The fourth instar at a corresponding size is much stouter due to the development of the thoracic imaginal buds and an accumulation of fat body. The fourth instar shows the rudiments of the pupal respiratory trumpets. The most prominent structure in this instar is the tail comb-spine.

Pupal biology - When the final moult approaches, larva becomes plump and increasingly turgid. The larva tends to cease feeding and to remain at rest at the surface. The pupal are comma shaped and white, but in a short time they show pigment changes. The pupal stage is quite short and usually last 1 to 2 days. The mosquito pupa is active, unlike pupa of the most insects.

Adult biology – *Aedes albopictus* breeds in temporary containers of several kind, generally prefers natural ones, such as tree holes, leaf axils, bamboo stumps, ground pools and coconut shells, and more often outdoors and less frequently indoors in man-made containers. Following its recent arrival in the western hemisphere, this species has increased its association with human populations by using discarded tires and vases in cemeteries as larval biotopes [17] (Figure 4). When females are exposed to long days are produced non-diapause eggs; exposure to short days results in the production of diapause eggs. Therefore, though diapause is expressed in the egg stage, the photoperiodically sensitive stage is the adult. Only *Ae. albopictus* strains from temperate climates are photoperiodic, whereas those from tropical or subtropical areas show no evidence of photoperiodicity [15].

The longevity of *Ae. albopictus* under natural environment is not fully known but it is expected to be shorter than under laboratory conditions. Laboratory studies showed that male and female *Aedes* mosquito survive an average of 20 to 30 days respectively (WHO, 1995).

Adult fly close to the ground and are not observed flying in strong winds [18]. Wind-aided long distance dispersal would thus be unlikely to occur in this species.

Aedes albopictus females have been collected biting both outdoors and indoors throughout its range, but the comparative data available indicate that this is an outdoor biting mosquito [15]. The time of peak biting activity varies with habitat. Though both an early morning and late afternoon peak of outdoor biting activity was noted by Wang [19] in China, indoor populations showed only a single broad period of activity in the afternoon. Mosquito Tiger bites at ground level, in particular on ankles and knees [20],

preferentially on warm-blooded animals and more rarely on reptiles [21]. It shows a plastic feeding behavior in its original range of distribution, and the same is observed in temperate areas, such as Italy [22]. Therefore is evident that this species has a high potential as vector of human pathogens in urban areas, where both humans and the mosquito itself may reach very high densities.

Figure 4 – Natural and artificial breeding sites for *Aedes albopictus*.

1.3. MEDICAL RELEVANCE

The receptivity of *Ae. albopictus* to pathogen viruses by laboratory experimental infection, as well as the list of viruses isolated from field-collected individuals is summarizes in Table 1. Included are the four quoted *Flaviviruses*, plus seven *Alphaviruses* and ten *Bunyaviruses*. One additional *Flavivirus* and two *Bunyavirus* have not been tested in the laboratory nor in the field but are known to circulate in the Mediterranean and to be pathogenic to humans [23].

Table 1 - Known virus receptivity in laboratory for *Ae. albopictus*, viruses isolated from wild mosquito populations, and human pathogenic viruses present in the Mediterranean [23-26].

		Laboratory Infection	Field Positive	Presence in the Mediterranean
Flaviviridae	Dengue (all 4 serotypes)	*	*	* (in the past)
	West Nile	*	*	*
	Yellow Fever	*		* (in the past)
	Japanese Encephalitis	*	*	
Bunyaviridae	Jamestown Canyon	*	*	
	Keystone	*	*	
	LaCrosse	*	*	
	Oropouche	*		
	Potosi	*	*	
	Rift Valley fever	*		*
	San Angelo	*		
	Trivittatus	*		
	Cache Valley	*	*	
	Tensaw		*	
	Tahyna			*
	Batai			*
Alphaviridae	WEE	*		
	EEE	*	*	
	VEE	*		
	Chikungunya	*	*	*
	Sindbis	*		*
	Mayaro	*		
	Ross River	*		

Dengue is important disease that can be transmitted of *Ae. albopictus*. It is a viral disease that has became an increasing public health problem in developing countries (Figure 5). Dengue is caused by any of four antigenically and genetically distinct viruses of the family *Flaviviridae*, named DEN-1, -2, -3 and -4.
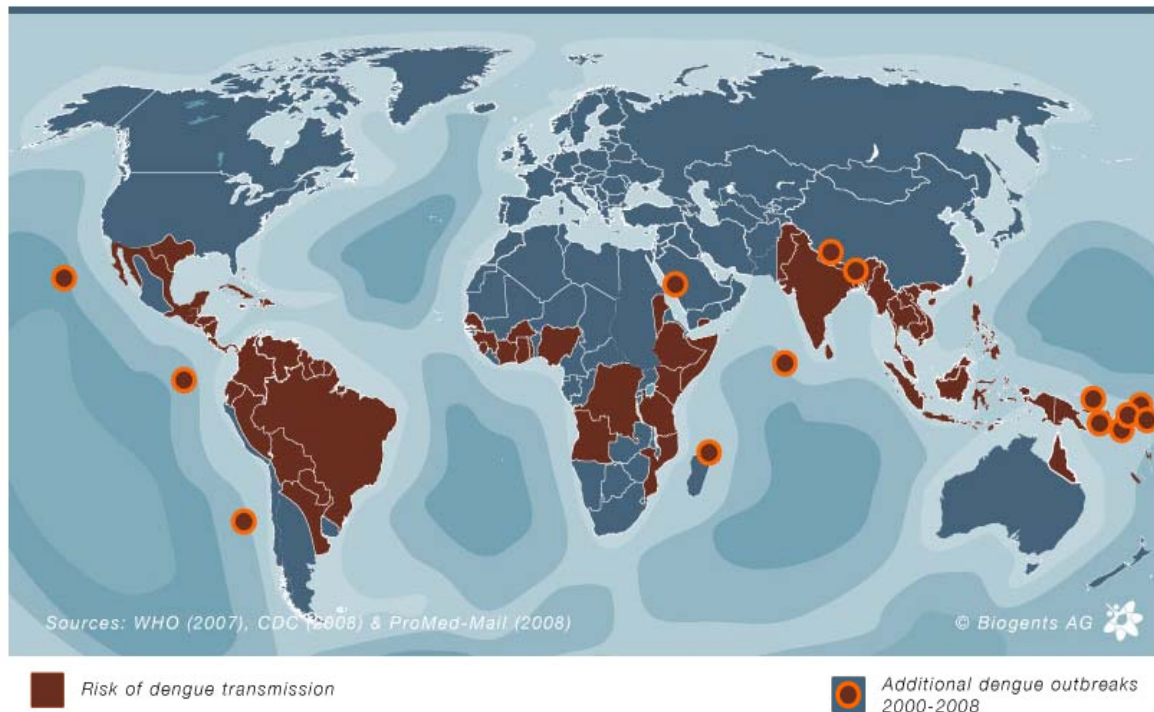
Figure 5 – Worldwide distribution of Dengue, 2008.

Humans are the primary vertebrate hosts of these viruses, although there is evidence of a silent zoonotic cycle involving mosquitoes and monkeys in parts of Asia and Africa. Dengue fever is characterized by an abrupt onset of fever, headache, myalgia, loss of appetite, and varying gastrointestinal symptoms, often accompanied by rash and bone or joint pains. Symptoms persist for 3–7 days, and while convalescence may be prolonged for several weeks, mortality is rare. Homologous immunity is lifelong, but cross-protection to other dengue viruses is not elicited and, indeed, there is evidence to suggest that heterologous antibodies may form infectious immune complexes which may increase the severity of subsequent infections. Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) are more severe manifestations of dengue infection, and are most often associated with infection of people with pre-existing antibodies to dengue, either actively or passively acquired. The clinical course follows that of classic dengue fever initially; however, as the febrile phase subsides, the patient's condition rapidly deteriorates with signs of circulatory failure, neurological manifestations, and hypovolemic shock with potential fatal outcome if prompt, proper, clinical management is not implemented. WHO currently estimates there may be 50-100 million cases of dengue

infection every year with about 500'000 DHF & DSS and about 100'000 death/year worldwide. *Ae. albopictus* is only second to *Ae. aegypti* in transmission of Dengue and it is believed to transmit the dengue disease in rural environments where human population density is much lower than in the cities, so that large outbreaks are not likely to occur [15]. In some cases, however, the absence of *Ae. aegypti* focuses on *Ae. albopictus* the responsibility for larger epidemics, such as the >100,000 cases outbreak in Japan during II World War [27]. Vazeille *et al*. [28] noted that *Ae. aegypti* does not transmit the virus vertically to its progeny very efficiently and thus may not contribute to the maintenance of the virus during interepidemic periods. On the other hand, transovarial transmission of Dengue has been demonstrated in the laboratory for *Ae. albopictus* [29] and has also been verified in field-collected larvae [25]. This mechanism could explain the maintenance of the virus in nature between epidemics in non-endemic areas where susceptible human or primate populations are not always present. Moreover, mosquitoes infected transovarially may transmit dengue virus to susceptible humans at their first blood-meal. European-established strains of *Ae. albopictus* from Albania [30] and Genoa, Italy [31], have been shown to be receptive to the virus.

Yellow fever (YF) is a other viral hemorrhagic fever transmitted by infected mosquitoes. It occurs only in Africa and South America. In South America sporadic infections occur almost exclusively in forestry and agricultural workers from occupational exposure in or near forests. Infection causes a wide spectrum of disease, from mild symptoms to severe illness and death. The "yellow" in the name is explained by the jaundice that affects some patients, causing yellow eyes and yellow skin. There are three types of transmission cycle: sylvatic, intermediate and urban. All three cycles exist in Africa, whereas in South America only sylvatic and urban YF occur. Sylvatic (or jungle) YF occurs in tropical rainforests where monkeys are infected by sylvatic mosquitoes. This produces sporadic cases, the majority of which are often young men working in the forest. The intermediate cycle of YF transmission occurs in humid or semi-humid savannahs of Africa, and can produce small-scale epidemics in rural villages. This is the most common type of outbreak seen in recent decades in Africa. Urban YF results in large explosive epidemics when travelers from rural areas introduce the virus into areas with high human population density. These outbreaks tend to spread outwards from one source to cover a wide area. The disease can be prevented by vaccination. When epidemics occur in unvaccinated populations, case-fatality rates may exceed 50%. No treatment beyond supportive care exists. *Aedes albopictus*, which has a great adaptive ability and in Southern America, represents an intermediate position between forest galleries where YF circulates and urban agglomerations where *Ae. aegypti* is present.

Chikungunya (CHIKV) activity in Asia has been documented since its isolation in Bangkok in 1958. Other countries which have reported chikungunya activity include Cambodia, Vietnam, Myanmar, Sri Lanka, India, Indonesia, the Philippines. The virus is transmitted in the savannahs and forests of tropical Africa by *Aedes* species of the

subgenera *Stegomyia* and *Diceromyia*. *Aedes aegypti* is an important vector in urban epidemics in both Africa and Asia. Chikungunya is an acute infection of abrupt onset, heralded by fever and severe arthralgia, followed by other constitutional symptoms and rash, and lasting for a period of 1-7 days. The incubation period is usually 2-3 days, with a range of 1-12 days. Fever rises abruptly and accompanied by intermittent shaking chills. This acute phase lasts 2-3 days. The temperature may remit for 1-2 days, resulting in a "saddle-back" fever curve. The arthralgias are polyarticular and predominantly affect the small joints of the hands, wrists, ankles and feet, with lesser involvement of larger joints. Patients with milder articular manifestations are usually symptom-free within a few weeks, but more severe cases require months to resolve entirely. Cutaneous manifestations are typical with many patients presenting with a flush over the face and trunk. Pruritis or irritation may accompany the eruption. Headache, photophobia and retro orbital pain also occur but not severe. Although *Ae. aegypti* has been the most efficient vector of CHIKV, during the last two years *Ae. albopictus* was responsible of chikungunya virus transmission in Comoros, Mauritius and La Reunion islands. The outbreak in La Reunion resulted in 255,000 suspected cases between March 1, 2005, and April 30, 2006, affecting >25% of the island's inhabitants. During this outbreak more than 250 people died [32]. Moreover, 205 cases of CHIKV infection occurred between July 4 to Sept 27, 2007, in Ravenna in Northern Italy (Figure 6). There were several waves of cases, with the number peaking in the third week of August. Up to the time of this peak, most cases had occurred in Castiglione di Cervia and Castiglione di Ravenna. Afterwards, and after the first mosquito control measures in the area that was mainly affected had been implemented (on Aug 18), a new wave of cases was observed, most of which occurred outside the two villages [33].

Ross river virus is endemic and epidemic in tropical and temperate regions of Australia. Large epidemics have been reported from Northern Territory, Queensland, Victoria, South Australia and New South Wales. Although never fatal, the discomfort and loss of productivity from joint symptoms persist for weeks and occasionally even years pose important public health problems. *Aedes* mosquitoes such as *Aedes vigilax* and *Aedes camptorhynchites*, and *Culex annulirostris, Mansonia uniformis* have been implicated as vectors. Nowdays, *Ae. albopictus* is spreading from Papua New Guinea to Australia through the Torres Straits islands [34] and is considered as a serious threat due to its capacity to transmit Ross river virus.

It is worth noting that *Ae. albopictus* may be a matter of concern as a bridge vector for West Nile because it inhabits rural areas [15] and has a wide host range including birds, so that it can pass enzootic cycles to humans. West Nile outbreaks have occurred in the Mediterranean but the 1996 outbreak in Romania was remarkable as 453 human cases occurred [35]. Following the introduction in 1999 of the virus in the US, during the single year 2002 a total of 4,161 human cases were reported, from which 277 died (CDC, unpublished data).

Figure 6 – Epidemic curve: distribution of dates of onset of symptoms for Chikungunya cases by presumed place of infection [33].

Aedes albopictus is also a vector of filariosis caused by *Dirofilaria immitis* [36]. In Italy there are been no evidences on the vector role of this specie until Cancrini *et al*. [37] detected the DNA of both *Dirofilaria repens* and *D. immitis* in a single *Ae. albopictus* collected in 2002 and considered that the species is a potential vector of both species of Dirofilaria in Italy. Cancrini *et al*. [38] carried out investigations in Padua town (Veneto region, NE Italy) to define the actual role of *Ae. albopictus* in the natural transmission of Dirofilaria. The results demonstrated that *Ae. albopictus* is a natural vector of *D. immitis* in Italy. The presence of the mosquito could affect the transmission pattern of canine heartworm disease in the urban environment and, considering the aggressive anthropophilic behaviour of the species (30–48 bites/h) in Padua, could enhance the circulation of filarial nematodes from animals to humans.

However in many temperate countries where *Ae. albopictus* is established, the medical importance of this species is due mainly to its ectoparasitic activity: in Italy, it has become the most serious pest mosquito species through most of its range [10].

1.4. DISPERSAL

Zoologists often disagree about the precise meaning of the words dispersal and migration [39-44]. Service [45] uses the word dispersal to describe all movements of adult mosquitoes, whether motivated or passive, and whether involving a few meters or hundreds of kilometers. The short distance dispersal is sometimes referred to as active dispersal to distinguish it from passive dispersal, which is applied to carriage by any other means, such as by wind, airplanes or ships.

Each mosquito population shows a different range of dispersal as a consequence of the species' intrinsic flight capability and its ecological setting. The active dispersal of adult mosquitoes is triggered by the need to find mates, sugar sources and resting sites and, in the case of females, hosts for bloodmeals and oviposition sites [45], therefore factors as the ecological nature of the release point, the distance to a blood meal, the distance to a water source for egg deposition and the distance to a natural resting place, can influence the rate of dispersion [18]. It is obvious that mosquitoes will be forced to fly greater distances than they normally would if released at a point distant from any or all of these enumerated items. Moreover the active dispersal depends on environmental thresholds, such as light intensity, wind speed and direction, and temperature, although winds may inhibit takeoff [46,47]. Wind is frequently incriminated as responsible for distribution, but it is of little account in the case of *Ae. albopictus*, which flies close to the ground and was never observed flying in a strong breeze. Adult mosquitoes were observed dropping in flight and clinging to grass or bush when moderate or heavy gusts of wind were present. In general, the only effect of wind is to orient the direction of flight, for this species was observed flying into the wind when his velocity were extremely low [18].

Knowledge about the movements of adult mosquito vectors in endemic or epidemic areas is valuable for understanding disease transmission dynamics and for determining the control limits necessary to interrupt pathogen transmission. During recent Chikungunya epidemics in the Indian Ocean islands in 2005 and 2006 [48] and in northeast Italy in 2007 [33] was highlighted the lack of this kind of information respect to a specie with wide spread, such as *Ae. albopictus*.

Since the discovery of the importance of mosquitoes as vectors of disease, numerous studies were carried out on the distances the various species travel from a breeding source or natural resting place. This distance has been variously named, "*flight*" [47,49], "*distance of flight*" [50], "*flight range*" [51], "*flying radius*" [52], "*range of dispersion*" [53]. Although mark-release-recapture (MRR) methods were primarily designed for the estimation of population size, most studies involving the marking of adult mosquitoes have been to measure dispersal. Different methods are available for marking adult mosquitoes in dispersal studies, such as stains, dusts, paints, radionuclides, ecc. Reiter *et al*. [54] have developed a method for marking eggs. They demonstrated that rubidium

dissolved in the blood meal is incorporated into the desiccation-resistant eggs of *Ae. aegypti* (Reiter P, Amador MA, unpublished data). Rubidium, a relatively rare alkali metal, is nontoxic to insects, animals, and plants. It has been used as a marker in studies of the movements and distribution of herbivorous insects and as a marker for mosquito blood. In field experiment, a solution (0.025 M) of rubidium chloride (RbCl) and fresh blood is used to fed to nulliparous females reared from eggs collected in the study area. Rb is incorporated into ovarian follicles and accumulates in mosquito eggs [55,56]. Eggs containing Rb were detected by atomic emission spectrophotometry [54].

The effectiveness of MRR studies is strongly affected by the quantity of marked specimens released, the ability to carry out recapture over a large study area and by several other confounding factors [46]. Moreover, the availability of an effective recapture method may represent a serious limitation in MRR studies. In the case of endophilic species, such as the main dengue vector *Ae. aegypti* (Diptera: Culicidae), marked mosquitoes can be efficiently recaptured by active aspiration in houses during their indoor resting phase [57], but this approach is much less efficient for collecting resting mosquitoes outdoors [58]. Thus, very little is known about the dispersal of exophilic species such as *Ae. albopictus* [18,59-63], despite its worldwide distribution [64] and its relevance as an arbovirus vector [13].

1.5. Aim of the Work

The objective of the present thesis was to study *Ae. albopictus* behavior in different ecological Italian contexts, to evaluate the her capacity of dispersion. Moreover it is evaluated the aptitude of the sticky-trap (ST, [65]) to be a sampling tool in this kind of studies. In fact, no information are currently available on several biological parameters (such as dispersion, longevity, exophily/endophly, host-preferences, ecc.) of *Ae. albopictus* in Italy, nor in other European temperate areas, despite their relevance in the evaluation of its potential vectorial role for human and animal pathogens and in the planning of control strategies.

During the first part of my PhD studies, I collaborated to study of the host preferences of *Ae. albopictus*, in urban and rural contexts within Rome province, Italy (Valerio L, **Marini F**, Bongiorno G, Facchinelli L, Pombi M, Caputo B, Maroli M, della Torre A, 2010. Host-feeding patterns of *Aedes albopictus* (Diptera: Culicidae)Vector-Borne and Zoonotic Diseases 10(3): 291-294). Successively, I carried out mark-release-recapture experiments to estimate the active dispersal of *Ae. albopictus* in a urban Italian context, as campus of University "Sapienza" of Rome, and consequently I have evaluated the effectiveness of the sticky trap (ST) as a recapture tool in this kind of protocols (**Marini F**, Caputo B, Pombi M, Tarsitani G, della Torre A, 2010. Studying *Aedes albopictus* dispersal in Rome (Italy) using sticky-traps in mark-release-recapture experiments. Medical and Veterinary Entomology 24(4): 361-8). Successively I have carried out the same experiments in a different ecological context, that periurban/rural of Piove di Sacco's Municipality, in province of Padua, north Italy (**Marini F**, Travaglio M, Pombi M, Caputo B, Neteler M, Montarsi F, Drago A, della Torre A. Analysis of the dispersal of *Aedes albopictus* in a rural-periuban context, in north Italy. In preparation.).

The data here presented refer to the results of field trials carried out in two different area, during the 2008 and 2009 field seasons.

These results provide the first data on the dispersal capacities of *Ae. albopictus* in temperate European areas and describe an effective methodological approach for MRR experiments focused on females.

2. MATERIAL AND METHODS

2.1. STUDY AREAS

Mark-release-recapture experiments (MRR) were carried out in two different ecological Italian contexts, urban and rural/peri-urban environment, respectively. The first area is represented to the campus of the University of Rome 'Sapienza' in central of Italy (RM), whereas the second is situated in Piove di Sacco's Municipality, in province of Padua in north Italy (PD).

`Campus of the University of Rome 'Sapienza' (RM)` - The campus of the University of Rome "Sapienza", that lies in the centre of Rome within a highly urbanized area, where high densities of *Ae. albopictus* have been reported [22] (Figure 7). The area is characterized by 10-20 m-high buildings (in grey, in Figure 7), separated by roads lanes and by opened and/or green areas. These consist mainly of either small flower-bed and lawn areas, or lanes bordered by trees or hedges. The whole area is surrounded by a ~3 m high wall interrupted by gates. Breeding sites are mostly represented by relatively homogeneously distributed drain-holes; flower pots and other water containers are also present.

Data on temperature were obtained from the Roma Porta Maggiore meteorological station, about 1.5 km from the release point.

The protocol received approval from the Rector of the University and extensive publicity among the University staff preceded the study.

`Piove di Sacco, province of Padua (PD)` - The study area is selected inside the Piove di Sacco's Municipality, it consists of a rural and peri-urban zone (Figure 8). The rural component is represented by extended crops of corn and alfalfa. Moreover, there are small vineyards and corrals whit animals to pasture (horses, sheep, poultry). Whereas the peri-urban zone is characterized by residential complexes (small houses on two level with garden), football and recreation grounds, stores, schools and a church. Finally there are many irrigation canals and the study area is surrounding to north and south by a wide road.

Data on temperature were obtained from the ARPAV agrometeorological station of Legnaro, about 10 km from the release point.

These experiments were approved and conducted in accordance with the Piove di Sacco's Municipality and the local population was informed the experiments.

Figure 7 - Campus of the University of Rome "Sapienza", in central Italy, with main buildings highlighted in grey, and the distribution of 55 sticky-traps (white dot) around the release sites (stars) for the three mark-release-recapture replicates (MRR1, MRR2 and MRR3).
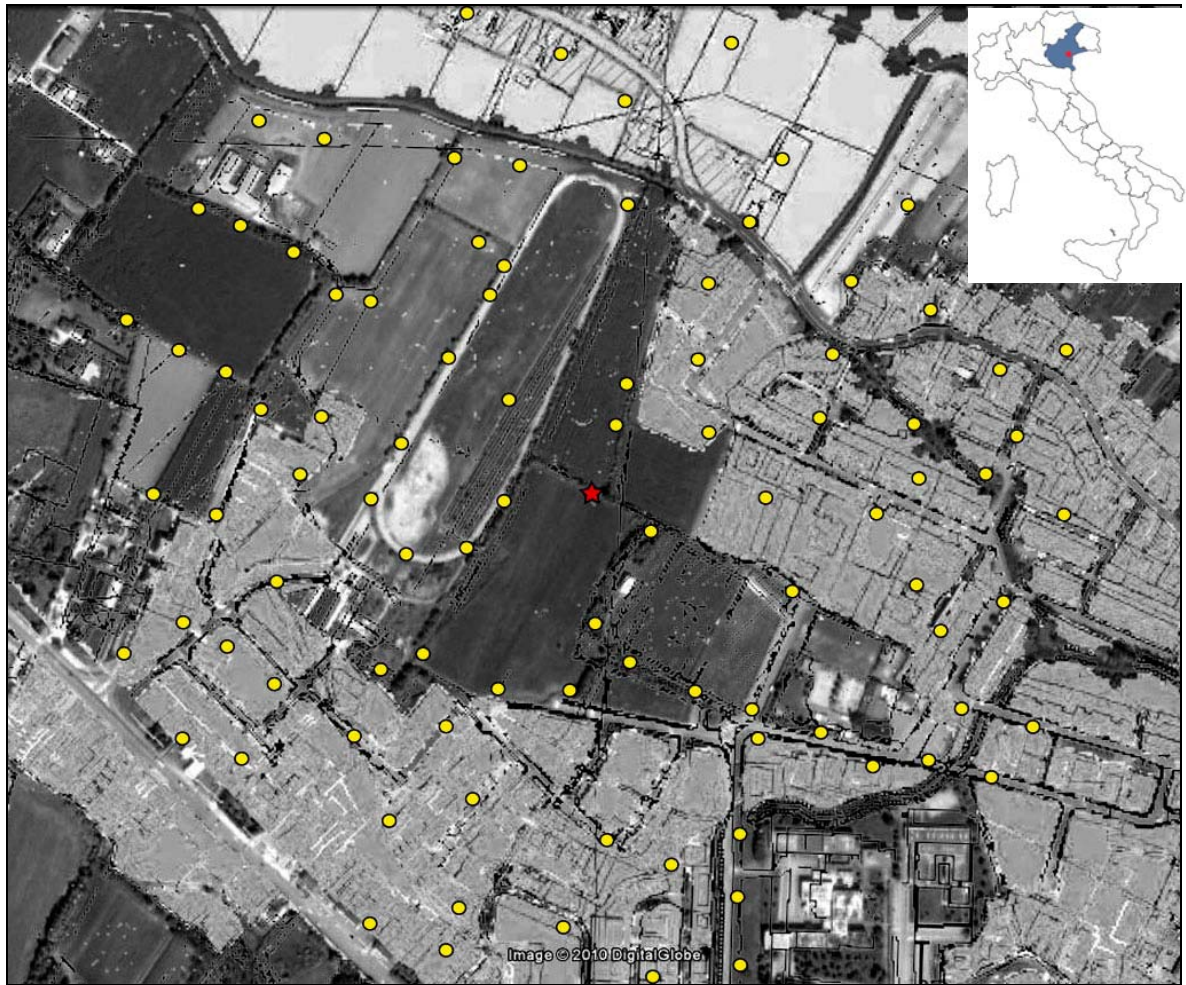
Figure 8 – Piove di Sacco, province of Padua, in north Italy, with the distribution of 96 sticky-traps (yellow dot) around the release site (red star) for the three mark-release-recapture replicates (MRR1, MRR2 and MRR3).

2.2. MARK-RELEASE-RECAPTURE PROTOCOLS

Aedes albopictus adults used for MRR experiments were obtained from eggs collected by ovitraps within the study area. Eggs were hatched and larvae reared to the adult stage in plastic basins (35 × 28 × 8 cm), with about 1 L of tap water and cat pellets as food, placed outdoors in a site sheltered from direct sunlight. Larval density was kept under 1 larva/mL. The pupae were collected and transferred to cubic cages (25×25×25 cm), where adults emerged and were maintained with 10% sugar solution for 2–9 and 2-4 days in RM and PD experiments, respectively. The mosquitoes were reared in semi-field conditions in both the areas, i.e. outdoors in sheltered sites. In RM, a wind protection was provided after some stress in the adults reared for the first MRR became apparent (i.e. most specimens rested on the bottom of the cage instead of on the walls).

On the morning of each release, males and females blood-fed by membrane feeders were separated and marked with orange fluorescent dust (Day-Glo Color Corp., Cleveland, OH, U.S.A.) by aspirating them into dusted paper cups (adult density: 10 individuals/cup). After 10 min, marked mosquitoes were counted and the paper cups were opened inside cages, which were later used to transfer the mosquitoes to the release sites. The possible effect of the marking procedure on mosquito survival was tested during each experiment, by comparing the mortality of marked and unmarked control individuals (handled as described above for the released specimens) and kept in separate cages that were left outdoors in sheltered sites up to end of each experiment.

The marked *Ae. albopictus* were released at the central point of study area at 1 h after sunset, when this specie is almost inactive [15], to minimize biases in the 'escape' dispersal caused by the release itself. Apparently unhealthy or dead adults found in the cages were counted and excluded from the total of released individuals. Table 2 shows the temporal intervals (start and end dates) during which each experiment was carried out.

Table 2 – Temporal intervals (start and end dates) during which each mark-release-recapture experiment (MRR1, MRR2 and MRR3) was carried out in Rome (RM) and Padua (PD).

	MRR1	13 Aug - 3 Sep 2008
RM	MRR2	9 - 30 Sep 2008
	MRR3	10 - 31 Oct 2008
	MRR1	14 Aug 2009
PD	MRR2	25 Aug - 9 Sep 2009
	MRR3	15 Sep - 1 Oct 2009

Adult mosquitoes were collected by sticky-traps (STs, Figure 9 [65]). In order to distribute the traps, the sampling area was virtually subdivided into concentric annuli of 50 m, increasing the number of STs from the release site to the extremes of the sampling

area (250 and 500 m radius for RM and PD, respectively). The STs were located at ground level in sheltered positions and and georeferenced using a global positioning system device (Garmin GPSMAP 60CSx). The particulars on sampling for each study area are shown in Table 3.

Note that because of problems in access, in the MRR-RM the release sites were not perfectly coincident in the three replicates (Figure 7; MRR1-RM: 41°54'10.46'' N, 12°30'52.62'' E; MRR2-RM: 41°54'10.73'' N, 12°30'53.47'' E; MRR3-RM: 41°54'9.57'' N, 12°30'51.27'' E), therefore also number of STs for each annulus change in three experiments (Table 3).

Figure 9 - Photographs showing details of the sticky trap used during mark-release-recapture experiments for collected adult mosquito.

Table 3 – Number of sticky-traps (STs) for annulus and sampling effort for mark-release-recapture replicates (MRR1, MRR2 and MRR3) in Rome and Padua (RM and PD), respectively.

ring	meters	N STs			
		RM			PD
		MRR1	MRR2	MRR3	MRR1, -2, -3
A	0-50	1	1	2	1
B	50-100	3	4	3	3
C	100-150	7	8	8	5
D	150-200	16	14	17	7
E	200-250	23	26	9	9
F	250-300	5	2	16	11
G	300-350	-	-	-	13
H	350-400	-	-	-	15
I	400-450	-	-	-	17
L	450-500	-	-	-	17
tot		*55*			*96*
sampling effort		≈1ST/3,600m^2			≈1ST/8,200m^2

2.3. MOSQUITO COLLECTION AND IDENTIFICATION

All STs were activated about 12 h after the release, except that in the centre of the sampling area, which was activated 5 days later in MRR-RM and next day in MRR-PD, to avoid the collection of individuals immediately after release (note that the 12 h between release and STs activation are indicated in the text and tables as day 0).

Periodically the STs were serviced as follows: (a) the sticky sheets were removed and brought to the laboratory; (b) the traps were emptied and equipped with new sheets freshly coated with glue, and (c) fresh tap water was added to approximately 500 mL/ST. While during the check daily each stuck *Ae. albopictus* was marked on the back of the adhesive sheets with a permanent marker, using different colors for each day, to allow the unambiguous determination of the day of capture. The monitoring scheme for each experiment in RM and PD is reported in Table 4.

Table 4 – Monitoring scheme (kind of activity and day on which it carry out) for each mark-release-recapture replicates (MRR1, MRR2 and MRR3) in Rome (RM) and Padua (PD), respectively.

		days after release	
		check to STs	service to STs
RM	MRR1	-	5, 9, 13, 17, 21
	MRR2	2, 3, 4, 7	
	MRR3		
PD	MRR1	2, 3, 4, 5	6, 11
	MRR2		6, 11, 16
	MRR3		

All mosquitoes collected were checked under ultraviolet light for the presence of fluorescent dust. Marked and unmarked mosquitoes were counted and morphologically subdivided by genus/species and gender under a dissecting microscope. In MRR-RM was determined the gonotrophic stage of marked mosquitoes collected, based on abdominal appearance (i.e. unfed, blood-fed, half-gravid and gravid).

2.4. TIMING OF OVIPOSITION

During the second and third MRR experiment in PD, the timing of oviposition of *Ae. albipictus* were investigated. Marked blood-fed females specimens (reared and handled as described above for the released) were kept in individual plastic cups (5.5x4x6 cm), containing filter paper soaked with water and placed in sheltered outdoors sites. Daily checks were carried out on the presence or not of eggs in each cup, from the day of release up to end of sampling in each MRR replica.

2.5. STATISTICAL ANALYSES

The effect of marking on mosquitoes was analyzed using the Kaplan-Meier method that constructs survival curves for unmarked (control) and marked cohorts, these were compared by Log-rank test.

Recapture rates were calculated for each MRR as the proportion of the number of recaptured marked mosquitoes over the total number of released ones and compared by Kruskal-Wallis test.

Dispersal of the released mosquitoes was calculated as the Mean Distance Travelled (MDT), a parameter which is not inherently biased for trap location or size of study area [66-68]:

$$MDT = \frac{\sum \left(ER \times median \quad dist \quad of \quad annulus \right)_{for \quad all \quad annuli}}{total \quad ER}$$

where ER is the number of recaptures that would be expected if the trap density was equal in each annulus:

$$ER = \frac{number \quad of \quad observed \quad recaptures \quad per \quad annulus}{number \quad of \quad STs \quad per \quad annulus} \times CF$$

where CF is a correction factor to account for differences in trap densities among annuli:

$$CF = \frac{area \quad of \quad the \quad annulus}{total \quad trapping \quad area} \times total \quad number \quad of \quad STs \quad in \quad total \quad trapping \quad area$$

MDT values were calculated for the several intervals of sampling and for full interval of each experiment. Moreover in some experiments, it was been possible calculate daily-MDTs for each of the first 5 days after release. Daily-MDTs among the replicates were compared by Mann-Whitney test.

Maximum distance travelled observed (MAX) was established as the linear distance from the release site to the most distant positive ST.

Flight ranges (FRs) were estimated from the linear regression of the cumulative number of expected recaptures (ERs) from each annulus (x axis) on the Log_{10} (annulus median distance +1). FR_{50} and FR_{90} values were calculated from the equation of regression line as 50% and 90% of the largest ER value, respectively [66-68].

The timing of oviposition of *Ae. albopictus* females was analyzed using Pearson's correlation, that compares the number of marked mosquito females collected during each replicate (MRR2-PD and MRR3-PD) and the number of the marked females that had laid eggs of the in individual plastic cups, during the same interval.

3. RESULTS

3.1.   CAMPUS OF THE UNIVERSITY OF ROME "SAPIENZA"

3.1.1.   SURVIVAL OF MARKED AEDES ALBOPICTUS

Survival in semi-field conditions was not affected by marking: there were no differences in survival between marked and unmarked females (MRR2-RM: log-rank test χ^2=0.6, df=1, p=0.44; MRR3-RM: χ^2=0.9, df=1, p=0.35, Figure 11), nor between marked and unmarked males (MRR2-RM: χ^2=1.1, df=1, p=0.30, Figure 10). The marking lasted the entire duration of the experiments on surviving control individuals.

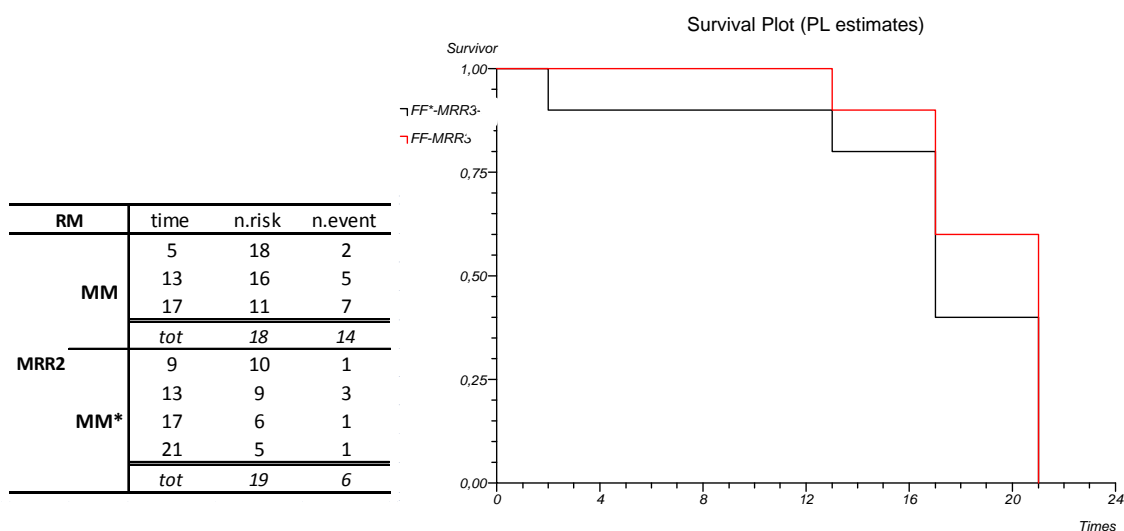| RM		time	n.risk	n.event
MRR2	MM	5	18	2
		13	16	5
		17	11	7
		tot	18	14
	MM*	9	10	1
		13	9	3
		17	6	1
		21	5	1
		tot	19	6

Survival Plot (PL estimates)

Figure 10 - In tables on left are reported the number (n.risk) of un-marked and marked *Aedes albopictus* males (MM and MM*, respectively) used to test the possible effect of the marking procedure on mosquito survival during the third mark-release-recapture experiment (MRR3) in Rome (RM). The number of mosquito dead in the sampling interval (time, expressed in day) is indicated as n.event. The graphic on right represents the survival curves for the two cohorts used for the test.
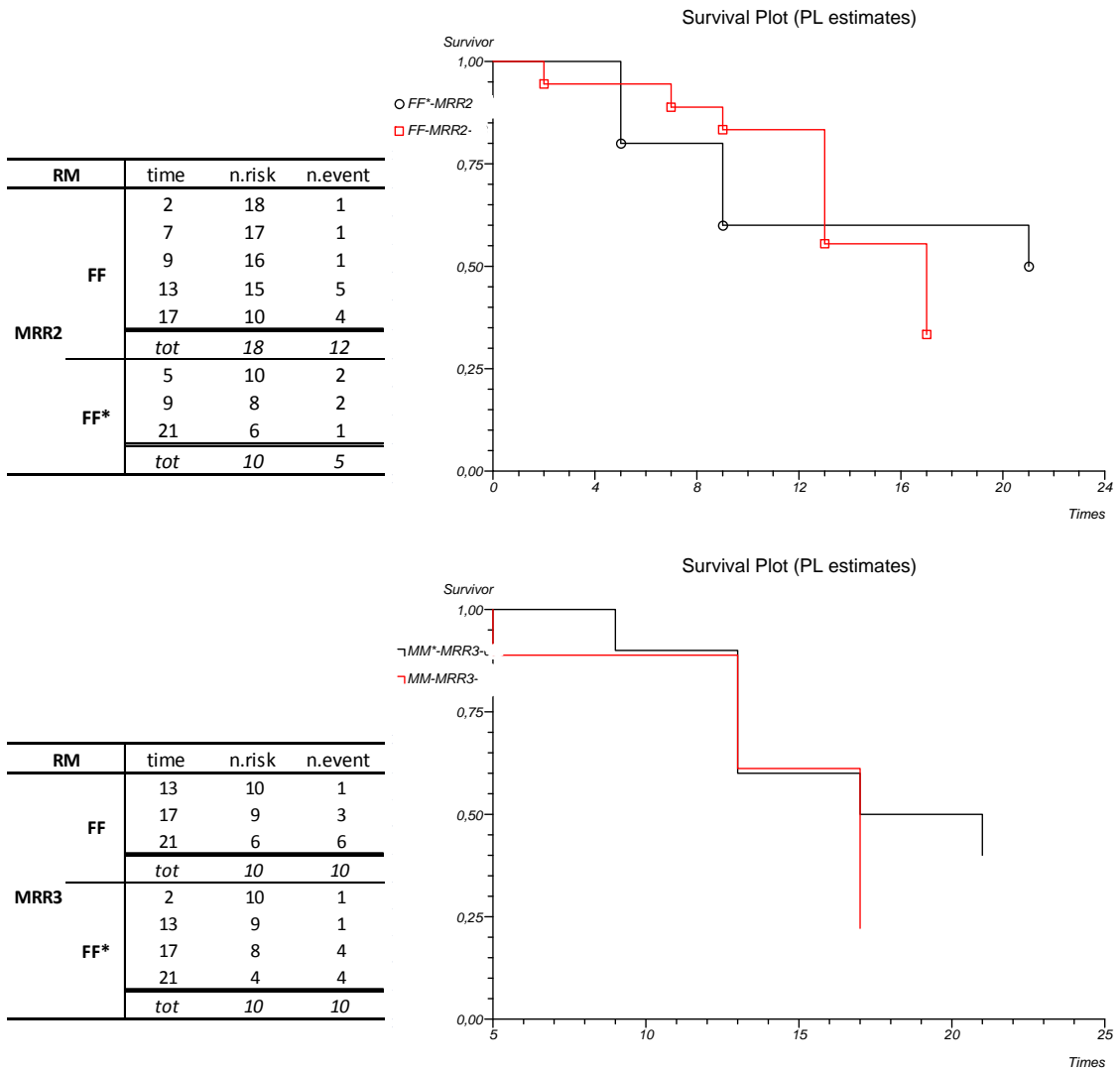
Survival Plot (PL estimates)

RM		time	n.risk	n.event
MRR2	FF	2	18	1
		7	17	1
		9	16	1
		13	15	5
		17	10	4
		tot	18	12
	FF*	5	10	2
		9	8	2
		21	6	1
		tot	10	5

Survival Plot (PL estimates)

RM		time	n.risk	n.event
MRR3	FF	13	10	1
		17	9	3
		21	6	6
		tot	10	10
	FF*	2	10	1
		13	9	1
		17	8	4
		21	4	4
		tot	10	10

Figure 11 - In tables on left are reported the number (n.risk) of un-marked and marked Aedes albopictus females (FF and FF*, respectively) used to test the possible effect of the marking procedure on mosquito survival during second (MRR2) and third (MRR3) mark-release-recapture replica in Rome (RM). The number of mosquito dead in the sampling interval (time, expressed in day) is indicated as n.event. The graphic on right rapresents the survival curves for the two cohorts used in each test.

3.1.2. *RELEASE AND RECAPTURE DATA*

In total, 464 (MRR1-RM), 566 (MRR2-RM) and 552 (MRR3-RM) marked females and 297 (MRR1-RM) and 506 (MRR2-RM) marked males, all of which had emerged directly from field-collected eggs, were released. No males were released in MRR3-RM. Released mosquitoes were aged 3–5 days, 6–9 days and 2–7 days in MRR1-RM, MRR2-RM and MRR3-RM, respectively.

A total of 3,055 *Ae. albopictus* females, 732 males and 133 specimens for which it was not possible to determine the gender were collected during the three replicates (1,345, 703, 1,007 females and 437, 163, 132 males in MRR1-RM, MRR2-RM and MRR3-RM, respectively). *Culex* mosquitoes, mostly females, were also found in the STs, but were not counted.

Table 5 shows the number of marked *Ae. albopictus* released and recaptured during each MRR replicate, subdivided following the monitoring scheme, which involved counting the collected individuals every 4 days in all replicates, and also on days 2, 3, 4 and 7 after release in MRR2-RM and MRR3-RM. It should be noted that, because of the 12-h interval between release and ST activation, days after release as indicated in the text and tables are reduced by 12 h (e.g. mosquitoes collected on day 2 were actually collected 12–36 h after release). Data on average, minimum and maximum temperatures during the three experiments are shown in Table 6. Rainfall was generally absent or scarce (<1 mm) except on a few days (MRR1-RM: 2 mm on day 2; MRR2-RM: <12 mm on days 3, 6 and 10; MRR3-RM: ≈4 mm on days 7, 20 and 21, and <20 mm on days 18 and 19).

The observed female recapture rates were 4.5% [95% confidence interval (CI) 3.0–6.8%] in MRR1-RM, 5.1% (95% CI 3.6–7.3%) in MRR2-RM, and 3.3% (95% CI 2.1–5.1%) in MRR3-RM. Fifty-five of 68 recaptured females were gravid (of which five showed traces of bloodmeal), seven were unfed, one was fed and the gonotrophic stage of five was not determined.

Male recapture rates were 1.3% (95% CI 0.5–3.4%) in MRR1-RM and 1.0% (95% CI 0.6–2.1%) in MRR2-RM. Recapture rates did not differ significantly among replicates, and the combined rates were 4.3% (95% CI 3.4–5.4%) and 1.1% (95% CI 0.6–2.1%) for females and males, respectively.

Overall, 81%, 90% and 61% of marked females were collected during the first 5 days of MRR1-RM, MRR2-RM and MRR3-RM, respectively. Significant differences were observed between the rate of recaptures over time in MRR2-RM and MRR3-RM: 22 of 28 females were collected between days 2 and 4 in MRR2-RM, whereas 15 of 17 females were captured between days 5 and 7 in MRR3-RM (χ^2=16.38, df=1, p<<0.001) (Figure 12).

Only one unfed female (aged 15–23 days) was collected more than 9 days after release in MRR3-RM. Among the few marked males recaptured, only two were collected after day 5 in MRR2-RM.

Table 5 – Number of marked *Aedes albopictus* females (males in parentheses) recaptured during the three mark-release-recapture replicates (MRR1, MRR2 and MRR3) in the concentric annuli around the release sites in Rome(RM).

		metres	♀♀(♂♂)							
			2 d	3 d	4 d	5 d	6-7 d	8-9 d	10-21 d	*tot*
RM	MRR1*	0-50	0 (0)				0 (0)		0 (0)	*0 (0)*
		50-100	7 (3)				2 (0)		1 (0)	*9 (3)*
		100-150	6 (1)				0 (0)		2 (0)	*6 (1)*
		150-200	4 (0)				2 (0)		3 (0)	*6 (0)*
		200-250	0(0)				0 (0)		4 (0)	*0 (0)*
		>250	0 (0)				0 (0)		5 (0)	*0 (0)*
		tot	*17 (4)*				*4 (0)*		*6 (0)*	*21 (4)*
	MRR2*	0-50	-	-	-	-	0 (0)	0 (0)	0 (0)	*0 (0)*
		50-100	2 (2)	4 (1)	1 (0)	2 (0)	1 (1)	0 (0)	0 (0)	*10 (4)*
		100-150	1 (0)	6 (0)	0 (0)	0 (0)	0 (0)	1 (0)	0 (0)	*8 (0)*
		150-200	2 (0)	2 (0)	1 (0)	1 (0)	1 (1)	0 (0)	0 (0)	*7 (1)*
		200-250	0 (0)	1 (0)	2 (0)	1 (0)	0 (0)	0 (0)	0 (0)	*4 (0)*
		>250	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	*0 (0)*
		tot	*5 (2)*	*13 (1)*	*4 (0)*	*4 (0)*	*2 (2)*	*1 (0)*	*0 (0)*	*29 (5)*
	MRR3*	0-50	0	0	0	1	0	0	0	*1*
		50-100	0	1	0	1	2	0	0	*4*
		100-150	0	0	1	2	1	0	0	*4*
		150-200	0	0	0	4	1	0	0	*5*
		200-250	0	0	0	1	1	0	0	*2*
		>250	0	0	0	0	1	0	1'	*2*
		tot	*0*	*1*	*1*	*9*	*6*	*0*	*1'*	*18*

*Number of marked females (males) released: MRR1:464(297); MRR2:566(506); MRR3:552(0)
'Collected between days 13 and 17 after release.

Table 6 – Average of mean daily temperatures (T), maximum (Max) and minimum (min) temperatures during the different sampling periods of the three mark-release-recapture replicates (MRR1, MRR2 and MRR3) in Rome (RM).

			2 d	3 d	4 d	5 d	6-7 d	8-9 d	10-21 d	*tot*
RM	MRR1	T	25.1				27.1		25.7	*25.8*
		Max	31.9				35.3		32.9	*35.3*
		min	19.3				20.6		19.6	*19.3*
	MRR2	T	27.5	26.0	22.6	21.1	18.8	19.7	17.4	*19.2*
		Max	34.4	33.8	25.6	24.1	25.4	26.7	25.2	*34.4*
		min	22.7	20.3	20.8	19.2	13.6	13.2	11.1	*11.1*
	MRR3	T	19.8	19.4	19.3	19.4	19.7	19.0	18.8	*19.1*
		Max	27.3	27.4	25.0	23.8	23.9	24.4	26.3	*27.4*
		min	14.2	13.6	15.3	15.9	16.8	14.6	13.7	*13.6*

All parameters are expressed in °C.
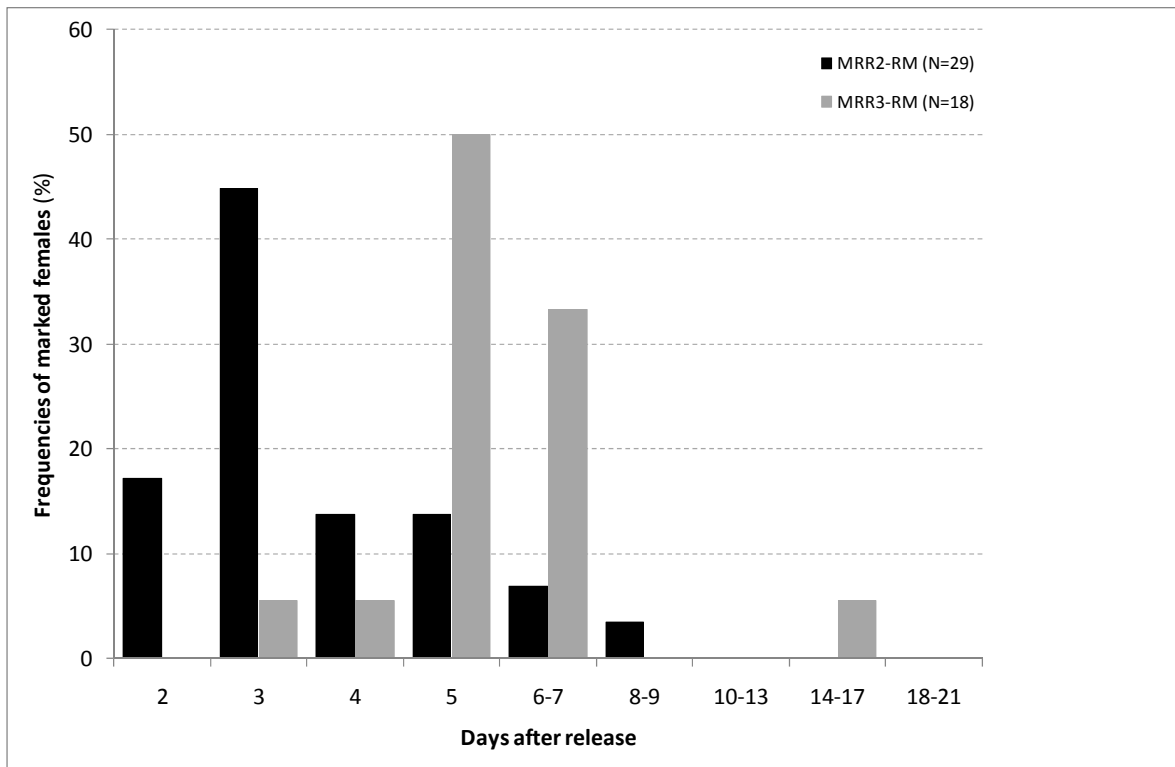d, days after release; tot, 2-21 days after release.

Figure 12 – Frequencies of marked *Aedes albopictus* females collected during the second (MRR2) and third (MRR3) mark-release-recapture replica in Rome (RM).

3.1.3.   DISPERSAL

Figure 13 shows the distribution of marked females in the STs in MRR1-RM, MRR2-RM and MRR3-RM and indicates that a large majority of females were recaptured at 50–200 m from the release sites. Only four females in MRR2-RM and four in MRR3-RM were found at a distance of >200 m (Table 5)

Table 7 shows the MDTs, MAXs and flight ranges of 90% (FR_{90}) and 50% (FR_{50}) of marked females in each replicate. The data refer to only the first 9 days after release, as only one female was collected after this period (MRR3-RM).

The cumulative MDTs (2–9 days) were 105 m, 121 m and 139 m in MRR1-RM, MRR2-RM and MRR3-RM, respectively (Table 7). Daily MDT values did not differ significantly between MRR2-RM and MRR3-RM (Mann–Whitney U=6, p=1; mean daily-MDT=119 ± 24 m). The MAXs were 199 m in 6–9 days, 230 m in 4 days and 290 m in 6–7 days in MRR1-RM, MRR2-RM and MRR3-RM, respectively (Table 7).

Most of the few marked males collected were found within the 100-m annulus (Table 5). The MAXs were 138 m in 2–5 days and 190 m in 6–7 days in MRR1-RM and MRR2-RM, respectively. Because of the low number of recaptured males, MDTs and flight ranges were not calculated.

Table 7 – Mean distance traveled (MDT) and maximum distance traveled (MAX) and flight rages of 90% (FR_{90}) and 50% (FR_{50}) of recaptured *Aedes albopictus* females in the three mark-release-recapture replicates (MRR1, MRR2 and MRR3) in Rome (RM).

	RM					
	MRR1		MRR2		MRR3	
	MDT	MAX	MDT	MAX	MDT	MAX
2 d			117	168	-	-
3 d	105*	164*	117	200	75	91
4 d			154	230	125	137
5 d			118	206	130	204
6-9 d	105	199	118	168	154	290
tot	*150*	*199*	*121*	*230*	*139*	*290*
FR_{90}	168		191		236	
FR_{50}	71		79		89	

All parameters are expressed in meters.
* Values corresponding to day 2-5 interval.
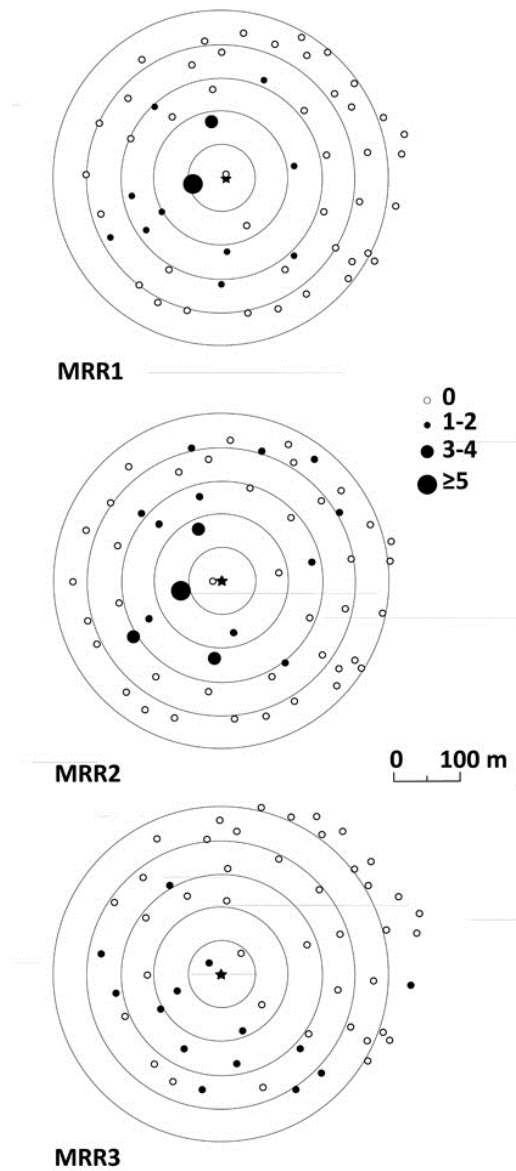d, days after release; tot, 2-21 days after release.

Figure 13 – Distribution of recaptured marked *Aedes albopictus* females in the sticky traps in the three mark-release-recapture replicates (MRR1, MRR2 and MRR3) in Rome, during the three sampling weeks.

3.2.   PIOVE DI SACCO, PROVINCE OF PADUA

3.2.1.   *SURVIVAL OF MARKED AEDES ALBOPICTUS*

Survival in semi-field conditions was not affected by marking: there were no differences in survival between marked and unmarked females (MRR1-PD: log-rank test χ^2=0.15, df=1, p=0.70, Figure 14; MRR2-PD: log-rank test χ^2=0.26, df=1, p=0.61; MRR3-PD: χ^2=0.10, df=1, p=0.76, Figure 15). The marking lasted the entire duration of the experiments on surviving control individuals.

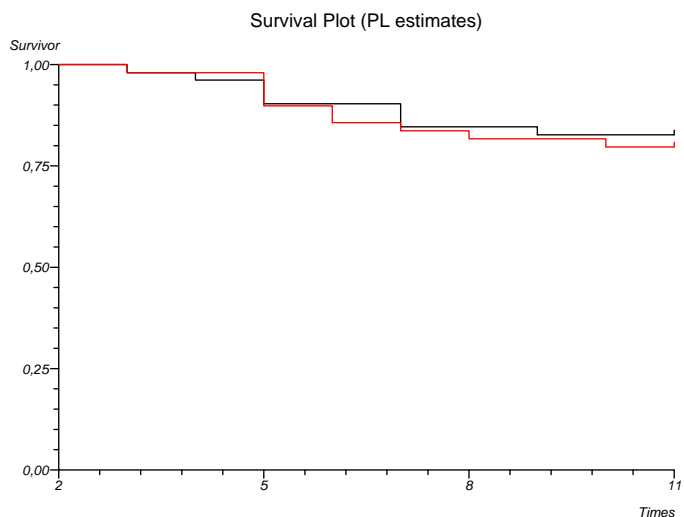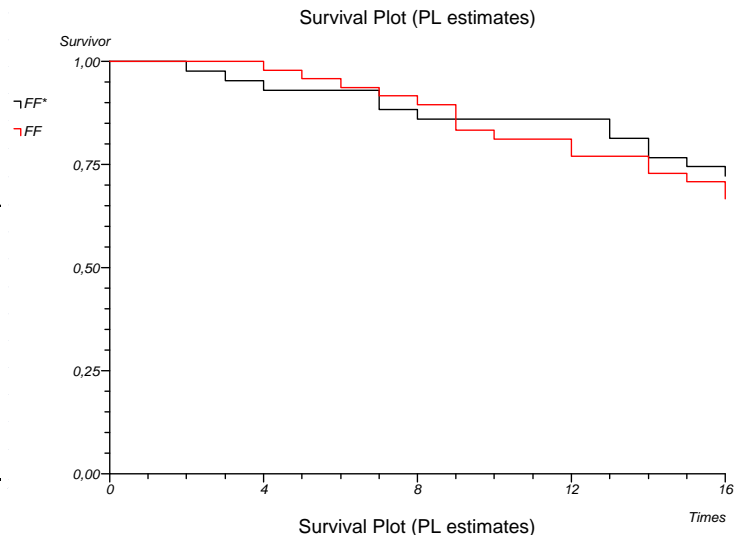| PD	time	n.risk	n.event
	3	49	1
	5	48	4
	6	44	2
FF	7	42	1
	8	41	1
	10	40	1
	11	39	0
MRR1	*tot*	*49*	*10*
	3	52	1
	4	51	1
	5	50	3
FF*	7	47	3
	9	44	1
	11	43	0
	tot	*52*	*9*

Figure 14 - In tables on left are reported the number (n.risk) of un-marked and marked *Aedes albopictus* females (FF and FF*, respectively) used to test the possible effect of the marking procedure on mosquito survival during first (MRR1) mark-release-recapture experiment in Padua (PD). The number of mosquito dead in the sampling interval (time, expressed in day) is indicated as n.event. The graphic on right represents the survival curves for the two cohorts used for the test.

PD		time	n.risk	n.event
		4	48	1
		5	47	1
		6	46	1
		7	45	1
		8	44	1
	FF	9	43	3
		10	40	1
		12	39	2
		14	37	2
		15	35	1
		16	34	2
MRR2		tot	48	16
		2	43	1
		3	42	1
		4	41	1
		7	40	2
	FF*	8	38	1
		13	37	2
		14	35	2
		15	33	1
		16	32	1
		tot	43	12

PD		time	n.risk	n.event
		8	48	1
		9	47	2
	FF	10	45	1
		13	44	2
		15	42	2
		16	40	1
MRR3		tot	48	9
		8	50	2
		9	48	1
	FF*	10	47	2
		13	45	1
		14	44	2
		16	42	0
		tot	43	8
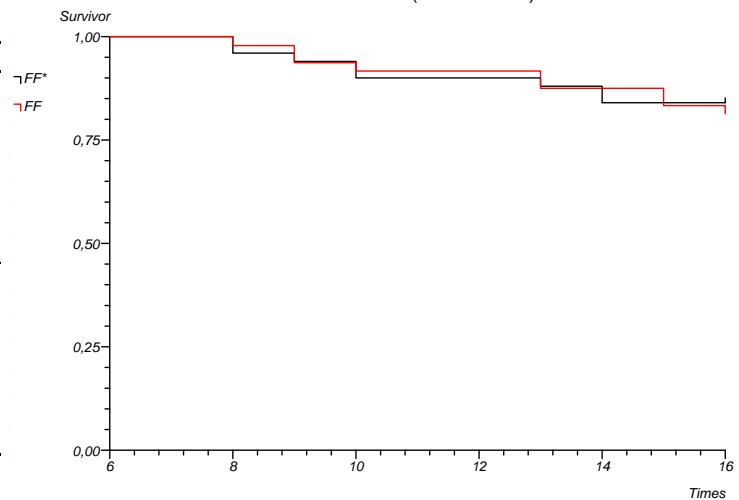
Figure 15 - In tables on left are reported the number (n.risk) of un-marked and marked *Aedes albopictus* females (FF and FF*, respectively) used to test the possible effect of the marking procedure on mosquito survival during second (MRR2) and third (MRR3) mark-release-recapture replica in Padua (RM). The number of mosquito dead in the sampling interval (time, expressed in day) is indicated as n.event. The graphic on right represents the survival curves for the two cohorts used in each test.

3.2.2.   RELEASE AND RECAPTURE DATA

In total, 1149 (MRR1-PD), 1600 (MRR2-PD) and 1210 (MRR3-PD) marked females, all of which had emerged directly from field-collected eggs, were released. Released mosquitoes were aged 1-4, 2-4 and 2-4 days in MRR1-PD, MRR2-PD and MRR3-PD, respectively.

A total of 13,782 *Ae. albopictus* females, 2,437 males and 1,076 specimens for which it was not possible to determine the gender were collected during the three replicates (3,708, 7,279, 2,795 females and 670, 1,533, 234 males in MRR1-PD, MRR2-PD and MRR3-PD, respectively). Some specimens of *Culex*, *Culiseta*, *Ochlerotatus caspius* mosquitoes, mostly females, were also found in the STs, but were not counted.

Table 8 shows the number of marked *Ae. albopictus* released and recaptured during each MRR replicate, subdivided following the monitoring scheme, which involved counting the collected individuals every 5 days in all replicates, and also on days 2, 3, 4 and 5 after release, up to day 11 in MRR1-PD and day 16 in MRR2-PD an MRR3-PD. Also in these replicates, as for those Rome, it should be noted that, because of the 12-h interval between release and STs activation, days after release as indicated in the text and tables are reduced by 12 h (e.g. mosquitoes collected on day 2 were actually collected 12–36 h after release). Data on average, minimum and maximum temperatures during the three experiments are shown in Table 9.

In MRR1-PD the rainfall was absent or scarce except on a few days (3-8 mm on days -10 and -11; 9 mm on day 7); whereas in MRR2-PD and MRR3-PD it was completely absent (<0.5 mm). Note that in MRR3-PD, in day of activation of the STs are fallen ~170 mm of rainfall.

The observed female recapture rates were 8.8% (95% CI 7.3–10.6%) in MRR1-PD, 13.4% (95% CI 11.8–15.1%) in MRR2-PD, and 3.4% (95% CI 2.5–4.6%) in MRR3-PD. The frequencies of marked *Ae. albopictus* females collected during the three mark-release-recapture replicates in Padua (Figure 16) were significantly different between themselves (Kruskal-Wallis test: H=7.66, df=2, p=0.02). In particular, the distribution of mosquitoes recaptured in MRR3-PD was meaningful different from that in MRR1-PD and MRR2-PD (Mann Whitney test: MRR1-PD *vs* MRR2-PD U=15, p=0.43; MRR1-PD *vs* MRR3-PD U=38, p=0.04; MRR2-PD *vs* MRR3-PD U=5.5, p=0.02). Marked *Ae. albopictus* female were collected for all sampling interval (2-11 days for MRR1-PD, 2-16 days for MRR2-PD and MRR3-PD, Table 8).

Table 8 - Number of marked *Aedes albopictus* females recaptured during the three mark-release-recapture replicates (MRR1, MRR2 and MRR3) in the concentric annuli around the release sites in Padua (PD).

		metres	2 d	3 d	4 d	5 d	6 d	7-11 d	12-16 d	tot
PD	**MRR1***	0-50	0	6	3	5	2	7	-	23
		50-100	1	9	8	12	8	10	-	48
		100-150	0	4	2	3	0	4	-	13
		150-200	0	0	1	0	1	0	-	2
		200-250	0	0	2	0	1	0	-	3
		250-300	0	1	0	0	0	2	-	3
		300-350	0	0	0	0	1	1	-	2
		350-400	0	0	1	0	0	1	-	2
		400-450	0	0	1	2	0	0	-	3
		450-500	0	0	2	0	0	0	-	2
		tot	1	20	20	22	13	25	-	101
	MRR2*	0-50	0	28	34	13	5	1	1	82
		50-100	3	7	25	12	7	1	7	62
		100-150	2	1	13	10	5	4	9	44
		150-200	0	1	2	3	0	0	1	7
		200-250	0	1	1	2	0	1	2	7
		250-300	0	1	2	3	0	0	1	7
		300-350	0	0	0	0	0	0	2	2
		350-400	0	0	1	0	0	0	0	1
		400-450	0	0	0	1	0	1	0	2
		450-500	0	0	0	0	0	0	0	0
		tot	5	39	78	44	17	8	23	214
	MRR3*	0-50	0	0	1	8	6	4	0	19
		50-100	0	0	2	2	8	2	1	15
		100-150	0	0	0	0	1	0	2	3
		150-200	0	0	0	0	0	1	2	3
		200-250	0	0	0	0	0	0	0	0
		250-300	0	0	0	0	0	0	0	0
		300-350	0	0	0	0	0	0	0	0
		350-400	0	0	0	0	1	0	0	1
		400-450	0	0	0	0	0	0	0	0
		450-500	0	0	0	0	0	0	0	0
		tot	0	0	3	10	16	7	5	41

* Number of marked females released: MRR1-PD:1149, MRR2-PD:1600; MRR3-PD:1210
d, days after release; tot, 2-16 days after release.

Table 9 - Average of mean daily temperatures (T), maximum (Max) and minimum (min) temperatures during the different sampling periods of the three mark-release-recapture replicates (MRR1, MRR2 and MRR3) in Padua (PD).

			2 d	3 d	4 d	5 d	6 d	7-11 d	12-16 d	tot
PD	MRR1	T	24.1	24.8	25.4	26.0	25.8	24.5		24.9
		Max	30.8	30.7	30.2	30.8	31.5	30.8	-	30.8
		min	16.3	18.0	19.9	22.0	20.3	19.3		19.3
	MRR2	T	24.9	26.7	27.2	25.4	21.7	23.8	20.3	23.1
		Max	30.1	32.1	35.1	31.1	26.5	31.1	25.6	29.2
		min	17.7	21.9	21.6	20.8	16.0	17.1	15.3	17.3
	MRR3	T	20.4	20.8	21.1	21.2	21.4	20.6	19.6	20.4
		Max	25.6	27.3	28.4	27.5	26.4	27.1	26.2	26.8
		min	16.8	15.8	16.1	16.3	17.6	15.3	14.2	15.3

All parameters are expressed in °C.
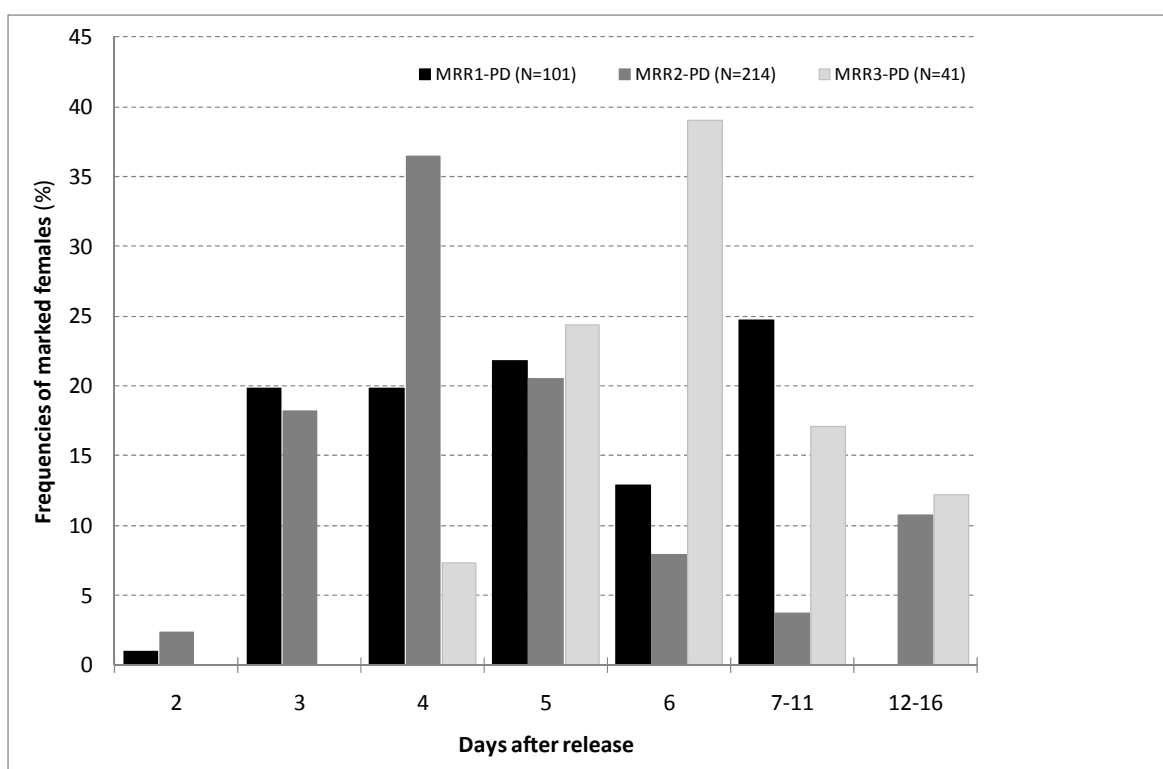d, days after release; tot, 2-16 days after release.

Figure 16 – Frequencies of marked *Aedes albopictus* females collected during three mark-release-recapture replicates (MRR1, MRR2, and MRR3) in Padua (PD). Note that in first experiment the sampling interval was 2-11 days, instead of 2-16 days as for other two replicates.

3.2.3.   TIMING OF OVIPOSITION

Two samples of 48 and 24 marked blood-fed females, respectively, were used to investigate on timing of oviposition of *Ae. albopictus*, during the sampling interval of MRR2-PD and MRR3-PD. In the first trial 36 mosquito had laid eggs in their cup and there was a positive correlation between the number of single ovipositions and the number of mosquito females recaptured (Spearman's rank correlation: r=0.92; t=5.38, df=5, p<0.01). Whereas in MRR3-PD, there were been 16 single oviposition, but there was not evidence of correlation between the two variables (Spearman's rank correlation: r=0.42; t=1.05, df=5, p=0.34). The Figure 17 shows the frequencies of marked *Ae. albopictus* females collected during each replicate (MRR2-PD and MRR3-PD) and the frequencies of the marked females that had laid eggs of the in individual plastic cups, during the same interval.
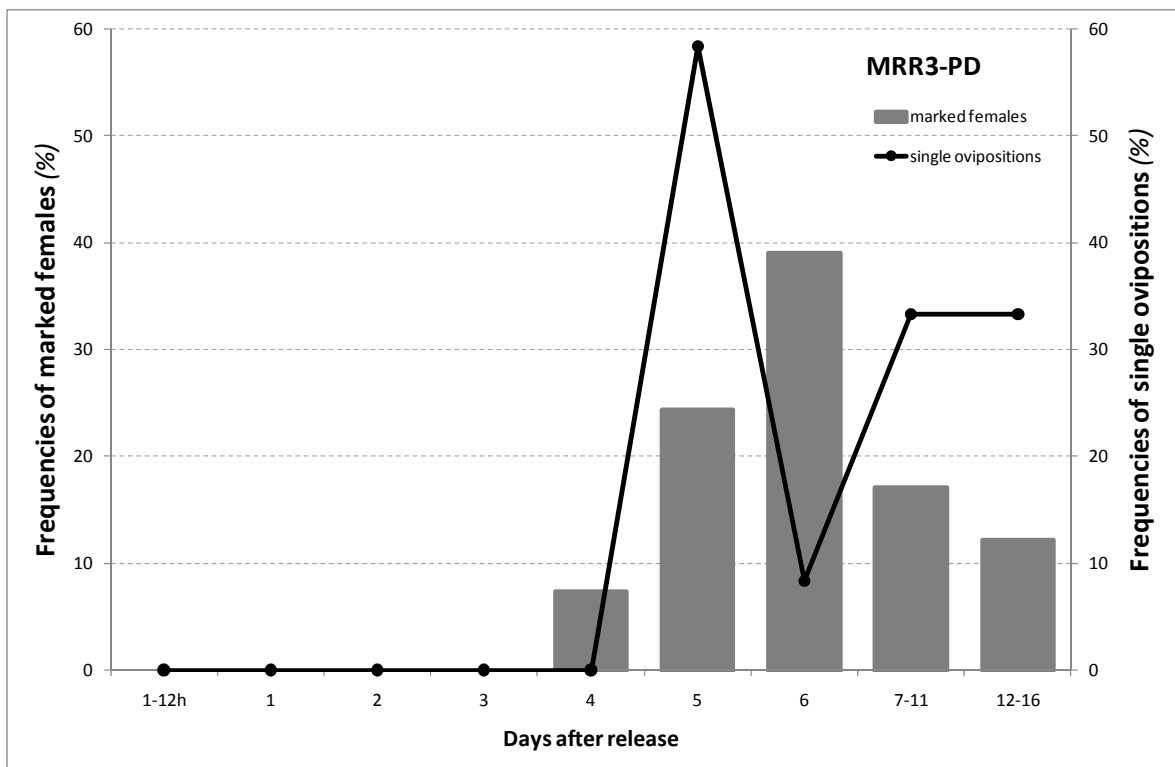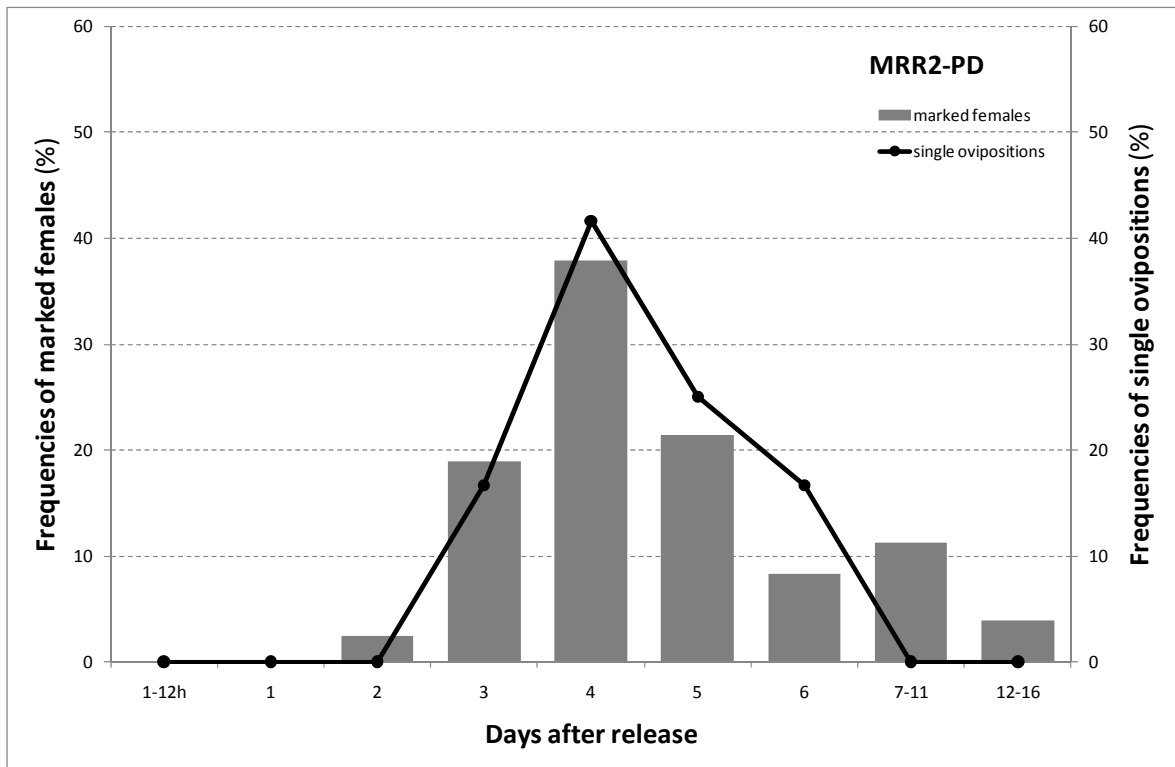
Figure 17 – Frequencies of marked *Aedes albopictus* females collected (grey bar) in the second (MRR2, upward) and third (MRR3, downward) mark-release-recapture replica in Padua (PD) *versus* frequencies of the marked females that had laid eggs of the in individual plastic cups (single ovipositions, black line) during the same interval.

3.2.4.   DISPERSAL

Figure 18 shows the distribution of marked females in the STs in MRR1-PD, MRR2-PD and MRR3-PD and indicates that a large majority of females were recaptured at 0–150 m from the release sites. Les of 20% (17, 12 and 10% in MRR1-PD, MRR2-PD and MRR3-PD respectively) of the marked mosquito were found at a distance of >200 m in all three the experiments (Table 8).

Table 10 shows the MDTs, MAXs and flight ranges of 90% (FR90) and 50% (FR50) of marked females in each replicate.

The cumulative MDTs (2–16 days) were 110 m, 77 m and 68 m in MRR1-PD, MRR2-PD and MRR3-PD, respectively (Table 10). Daily MDT values did not differ significantly between MRR1-PD and MRR2-PD (Mann Whitney U=27.5, p=0.19), even if they were statistically different of the daily MDT values of the MRR3-PD (Mann Whitney test MRR1-PD *vs* MRR3-PD U=4, p=0.03; MRR2-PD *vs* MRR3-PD U=1, p=0.01).The MAXs were 464 m in 4 days, 433 m in 5 days and 375 m in 6 days in MRR1-PD, MRR2-PD and MRR3-PD, respectively (Table 10).

Table 10 - Mean distance travelled (MDT) and maximum distance travelled (MAX) and flight renges of 90% (FR_{90}) and 50% (FR_{50}) of recaptured *Aedes albpoicuts* females in the three mark-release-recapture replicates (MRR1, MRR2 and MRR3) in Padua (PD).

	PD					
	MRR1		**MRR2**		**MRR3**	
	MDT	MAX	MDT	MAX	MDT	MAX
2 d	75	74	95	115	-	-
3 d	80	287	46	275	-	-
4 d	171	464	72	388	58	71
5 d	104	435	97	433	35	71
6 d	106	332	75	132	78	375
7-11 d	107	355	121	332	25	169
12-16 d	-	-	132	433	25	193
tot	*110*	*464*	*77*	*433*	*68*	*375*
FR90	252		209		177	
FR50	50		31		21	

All parameters are expressed in meters.
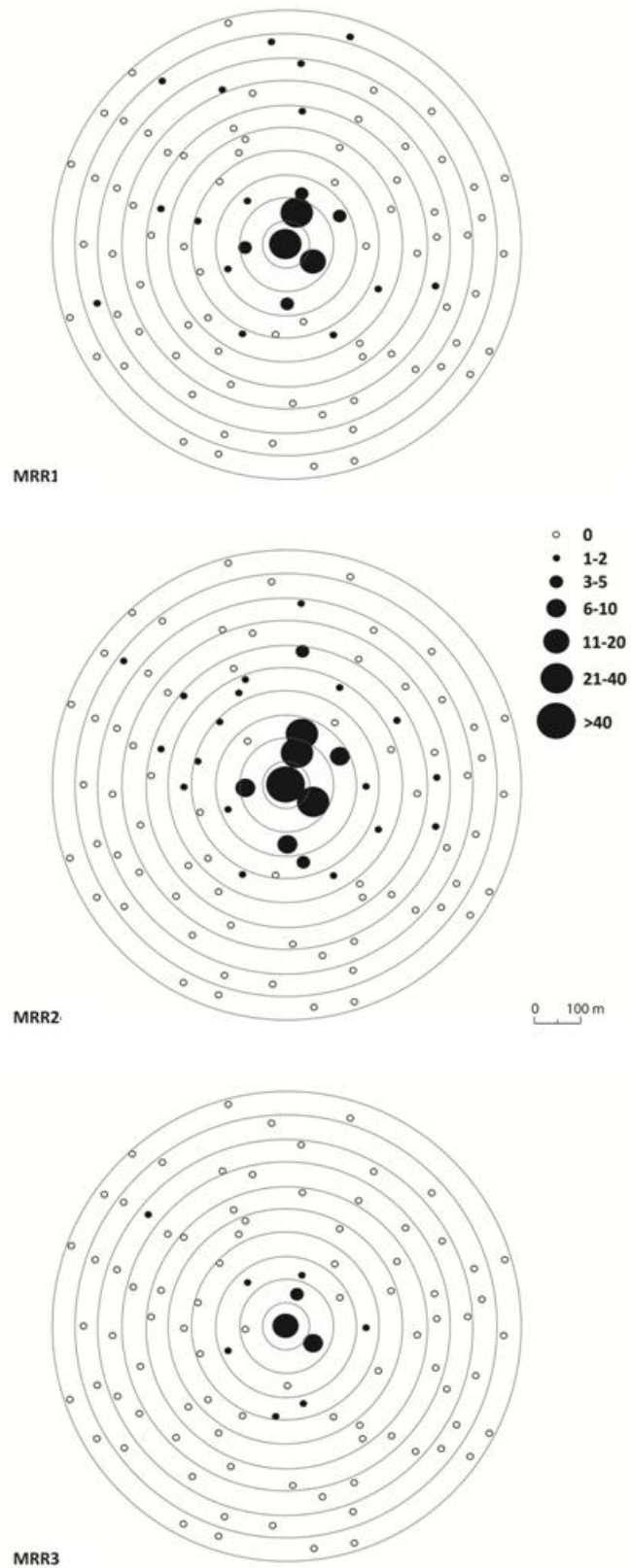d, days after release; tot, 2-16 days after release.

Figure 18 - Distribution of recaptured marked *Aedes albopictus* females in the sticky traps in the three mark-release-recapture replicates (MRR1, MRR2 and MRR3) in Padua, during the sampling interval (2-16 days after release).

4.DISCUSSION

`Recapture rates` – The recapture rates of marked *Ae. albopictus* females in the 3 replicates of the 2 MRR experiments ranged between 3.3 and 5.1% in RM and 3.4 and 13.4% in PD. These rates allow to infer the first parameters pertaining to the dispersal of females of this species in temperate European areas.

Direct comparisons with recapture rates obtained in the few studies evaluating *Ae. albopictus* female dispersal by MRR experiments are tricky as a result of differences in the recapture approaches used and in experimental conditions (e.g. trap densities, ecological and climatic conditions). However, it is noteworthy that a similar recapture rate was obtained in the only MRR study exploiting sticky ovitraps to recapture *Ae. albopictus* (6.1% in Brazil [62]). Moreover, the rates here reported are in the range of those obtained in most MRR studies utilizing different kinds of sticky traps to collect marked *Ae. aegypti* females in diverse ecological settings (i.e. 2.7–8.7% in Australia, with sticky lures [69]; 7.7% in Mexico, with sticky ovitraps [70]; 19–26% in Morea, French Polynesia, with sticky ovitraps baited with water or grass infusions rather than leaf infusions [71]; 3.4% in Queensland, Australia, with sticky ovitrap [72] and 6.4% in Brazil, by MosquiTRAPs baited with attractant [73]). In other MRR studies aimed at evaluating *Ae. albopictus* female dispersal, various rates of recapture were obtained by different recapture approaches, including: 3.8% by human landing catches in Hawaii [18]; 8.1% by vacuum aspiration at a scrap tire yard and in vegetation in Missouri, U.S.A. [59], and 24.4–30.6% by mouse-baited BG-sentinel traps in La Réunion Island [63]. It should be noted that these studies were very labor-intensive because 'active' recapture approaches were utilized (e.g. two 3-h trapping sessions/day with 20–28 BG-sentinel traps [63]). By contrast, the ST represents a 'passive' recapture approach with peculiar characteristics. Firstly, a high density of STs is feasible with relatively limited effort in terms of labor and costs. In fact, the STs (55 in RM and 96 in PD) were serviced by a single person in RM and by two persons in PD, in 5–6 h when the adhesive sheets were replaced. Secondly, the ST collects mosquitoes continuously over time, not at defined time-points, and the numbers of specimens trapped are not affected by the timing of aspiration and/or the skill of the collectors. Thirdly, the ST mostly captures gravid females (although the range of females unfed captured is between 24% and 27% in a urban and rural area, respectively [74]) and, thus, focus on a different fraction of the female population compared to active aspiration and mouse-baited BG-sentinel traps, which mostly capture resting and host-seeking mosquitoes, respectively. Moreover, focusing on gravid females is also advantageous because blood-fed, rather than host-seeking, individuals can be released, thus decreasing the nuisance level in the proximities of the release sites. The main limitation of the ST is that its collection efficiency is biased by competition with natural oviposition (and resting) sites, the density of which in each study area is inversely correlated with the ST's power

of collection [65]. This implies that, unless detailed information on the relative abundance of natural oviposition sites in the study area is available, caution must be applied when comparing numbers of mosquitoes collected in STs located in different areas in order to estimate differences in either mosquito densities or flight direction in the case of MRR experiments.

A total of 87% of the females recaptured in MRR experiments in RM were gravid and traces of blood-meal were found in only five of them. We hypothesized that females were mostly trapped when searching for oviposition (rather than resting) sites, and assumed that most of them had completed a single gonotrophic cycle triggered by the blood-meal provided before releases. This is confirmed by the significant correlation observed between the frequencies of marked females recaptured along time in MRR2-PD and the frequencies of single female oviposition observed under semi-natural condition during the same day interval (Figure 17). In this case, the 2-day shift in the peaks of recaptures of marked females along time observed in the third replicates compared to the previous ones both in RM and in PD (Figure 12 and Figure 16), could be attributed to an increase in the length of gonotrophic cycles due to lower temperature at the end of the summer season.

In MRR3-PD, however, no correlation was found between the frequencies of marked females recaptured along time and the frequencies of single female oviposition observed under semi-natural condition. This could be attributed to a more significant impact of adverse climatic conditions (i.e. lower temperatures and heavy rain) on the length of the gonotrophic cycle of released females as opposed to that of females maintained under semi-natural conditions to evaluate the timing of oviposition.

Recapture rates for marked *Ae. albopictus* males were 1.0 and 1.3%. in the two replicates carried out in RM. Due to these low recapture rates male's dispersal was not analyzed in the third replicate in RM, nor in MRR carried out in PD. The above rates were lower than those obtained in the only other two MRR studies aimed at evaluating *Ae. albopictus* males dispersal. Lacroix *et al*. [63] obtain rates ranging between 6.4–15.8% by mouse-baited BG-sentinel traps in La Réunion Island; a overall recapture rate (males and females) of 8.1% was observed by vacuum aspiration human-bait landing catches in Missouri, USA [59]. However, this result was expected, because the ST is a collecting tool focused on females rather than males, as show by the fact that wild males collected during the 2 MRR replicates were 22% of the total number of *Ae. albopictus* collected.

`Dispersal` – Articles on *Ae. aegypti* dispersal [69,72,73,75,76] report statistically calculated parameters, such as mean and/or maximum observed distances travelled (i.e. MDT and MAX) and the flight ranges for 50% and 90% of recaptured females (i.e. FR_{50} and FR_{90}), which have never been calculated in the case of *Ae. albopictus*. We here provide the first estimation of these parameters for this species. The calculated values were quite homogeneous for each replicate in RM, allowing to determine a mean daily MDT of 119

m, whereas in MRR-PD experiments, the third replica was significantly different from other two. The MAX distance traveled observed ranged from 199–290 m in RM and 375–464 m in PD, respectively, meaning that in both sites females reached the limit of the study areas. In fact, single females flew up to 230 and 464 m in 4 days in RM and PD, respectively. FR_{50} values were 71-89 and 21–50 m in RM and PD, respectively. The higher values observed in RM, are due to the fact that no females were recaptured in the single ST within the 50 m annulus, likely as a consequence of a lower suitability for resting of the area around the release site (i.e. an concrete area with little vegetation). In fact, the ST in the PD release site, located in a wilder and very green area, close to trees and bushes, collected several marked females. On the contrary, FR_{90} values were quite homogeneous in the two study areas (i.e. 168–236 m and 177–252 m in RM and PD, respectively), despite that the differences in the size (250 m *versus* 500 m radius in RM and PD, respectively) and ecology (urban *versus* peri-urban/rural) of the two sampling areas.

The range of dispersal of *Ae. albopictus* females while searching for oviposition sites was previously investigated by MRR and ovitrap collections of rubidium (Rb)-labelled eggs in Brazil [60,62] and in Singapore [61]. These studies provide only rough estimation on the percentage of females recaptured at different distances from the release sites. In RM and PD most females were recaptured at 50-200 m and 0-150 m from the release sites, respectively. These ranges are not very different from those obtained in the above studies carried out in urban settings, despite the fact that Rb-based approach allows females to visit several ovitraps while scattering eggs (thus giving only an inference of the actual number of mosquitoes laying eggs in each ovitrap), whereas a female visiting an ST is immediately captured. Hoñorio *et al*. [60] reported that, in an urban area in Brazil, the highest frequency of Rb-marked eggs occurred in the 100–200 m annulus around the release site, although marked eggs were also found up to 800 m distant from the site. Liew & Curtis [61] observed Rb-marked eggs in ovitraps throughout the urban area sampled (320 m in radius), but no significant variations were observed at different distances from the release point. A much higher dispersal range was, however, observed for *Ae. albopictus* Rb-marked eggs in a Brazilian area encompassing forested and urban sites [62]. In this case, MRR experiments based on both collections of Rb-marked eggs and recapture of fluorescent dust-marked females by sticky ovitraps were carried out. Both approaches showed that females released in the forest could travel over 1000 m to reach an urban area with high densities of possible hosts, whereas, when they are released in a peridomestic environment, most of them remain close to houses in suburban and rural vegetated areas. The above evidence clearly shows that results obtained from MRR experiments cannot be generalized because they depend on the ecological characteristics of the study sites. Moreover, the ecological factors affecting dispersal vary depending on the objectives of the mosquito dispersion (i.e. host seeking, resting or oviposition site seeking), which, in turn, imply different recapture approaches. Thus, it is not possible to compare the dispersal of females searching for oviposition sites with that estimated for

host-seeking or resting females. However, it is again interesting to note that most marked females were recaptured within either 50 m from the release site by BG-sentinel traps in a rural area in La Réunion Island [63], or 100 m by landing catches in a rural area in Hawaii [18], or by aspiration at a scrap tire yard and in vegetation in Missouri [59].

It is important to highlight that MRR studies underestimate the actual flight of the mosquitoes because they measure linear distances from the release to the collection sites, whereas mosquitoes probably follow indirect routes in order to overcome physical barriers, such as buildings, dense vegetation, etc. For instance, it is likely that the unequal distribution of blocks of buildings and of green areas may have determined the apparently unequal distribution of recaptured females in our two study areas (Figure 7 and Figure 8). In fact, lower values than those we observed for *Ae. albopictus* were observed in sticky trap MRR studies on *Ae. aegypti* in situations where dispersal was constrained by physical and/or geographical barriers, but these values became much higher in unconstrained situations ([73] and references therein).

5.CONCLUSIONS

This study represents the first evaluation of the dispersal of *Aedes albopictus* females in Europe. Despite the ecological differences between the two study areas (i.e. the university campus in Rome and the study site in Padova, taken as representative of urban a periurban/rural areas, respectively) and between the two study designs, we obtained quite similar estimates of the population flight ranges of fed females looking for oviposition sites after a single gonothrophic cycle. These ranges were showed to be below 100 m for 50% of individuals in each population, and between 170 and 250 m for 90% of them. The released females were able to rapidly disperse over the two study areas, even under limited "pressure" (i.e. a relatively high abundance of oviposition sites and hosts) and single females were shown to have flew up to 464 m in only 4 days. It is, thus, possible to hypothesize that the *Ae. albopictus* flight range might increase under more intense "pressures" (e.g. low abundance of oviposition sites or hosts for blood-meals).

These conclusions are particularly instrumental for the planning of control activities in Italy, as well as in other European countries, and for determining of appropriate control limits to interrupt the transmission of pathogens in cases of possible arbovirus epidemics, such the Chikungunya outbreak that occurred in Emilia Romagna in 2007. It is in fact interesting to note that following this outbreak, the Emilia Romagna region published a leaflet including guidelines for control intervention to prevent other outbreaks [77], where it is suggested that the insecticides treatments should be focused to a 100-m radius area around the suspected infected patient. Our results suggest that this limit may be significantly lower than that needed to efficiently prevent a possible outbreak.

Finally, we demonstrated that ST represents an useful tool, not only for studies aimed to monitor *Ae. albopictus* population dynamics or host preferences, but also in the frame of MRR experiments focused on analyzing the dispersal of the females of these species or of other exophilic container-breeding species. Moreover, although the ST is not very efficient in collecting males, the information obtained on the dispersal of females may also be relevant for the planning of strategies aimed to reduce *Ae. albopictus* densities by means of the mass releases of sterile males, an approach currently under consideration for highly infested areas in Italy [78].

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PUBLICATIONS & PRESENTATIONS (2008-2010)

The publications and presentations to national/international meetings originated during the period of PhD are as follows:

PUBLICATIONS:

MARINI F, TRAVAGLIO M, POMBI M, CAPUTO B, NETELER M, MONTARSI F, DRAGO A, DELLA TORRE A. Analysis of the dispersal of *Aedes albopictus* in a rural-periuban context, in north Italy. *In preparation.*

MARINI F, CAPUTO B, POMBI M, TARSITANI G, DELLA TORRE A, 2010. Studying *Aedes albopictus* dispersal in Rome (Italy) using sticky-traps in mark-release-recapture experiments. Medical and Veterinary Entomology 24(4): 361-8.

VALERIO L, **MARINI F**, BONGIORNO G, FACCHINELLI L, POMBI M, CAPUTO B, MAROLI M, DELLA TORRE A, 2010. Host-feeding patterns of *Aedes albopictus* (Diptera: Culicidae) in urban and rural contexts within Rome province, Italy. Vector-Borne and Zoonotic Diseases 10(3): 291-294.

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MARINI F & DELLA TORRE A, 2010. Nuovi approcci allo studio della biologia di *Aedes albopictus* in Italia: risultati e prospettive. Workshop "Vector borne diseases and global health" (Venezia, Italia 14-15 Giu 2010).

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PRESENTATIONS AND POSTERS DURING NATIONAL/INTERNATIONAL MEETINGS:

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