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Public health issues posed by mosquitoes

An independent report

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Contents

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This independent report was commissioned by the Chartered Institute of Environmental Health as part of the debate on the need for greater public health interventions in resolving pest problems. Its views are not necessarily those of the CIEH.

1.0 Introduction

In Britain, the seemingly fanciful scenario of widespread cases of protracted debilitating illness and loss of life due to epidemics of malaria is in fact an ancient historical reality that persisted until the late 19th century. The last major outbreak of indigenous transmission is still within living memory and resulted from the large number of malaria infected soldiers returning after the First World War (Dobson, 1994).

Despite dire predictions from global warming and about 60,000 imported malaria cases over the last half century, recent analyses based on computer generated models suggest the risk of a return to those days is remote (Hutchinson & Lindsay, 2006;Kuhn *et al*., 2003;Lindsay & Joyce, 2008;Reiter, 2000). Models of this type are only best guesses, but whether they are accurate or not, it would be foolhardy to equate estimates of low risk with no risk.

British mosquitoes, including known malaria vectors, are a largely unacknowledged public health issue that is once again increasing in importance: ranging from localised biting nuisance to justifiable concerns that there will be an outbreak of some form of mosquito borne disease.

The latter should not overshadow the significance and extent of biting, which is much more than just an inconvenience to those being attacked and can lead to psychological and medical problems, in some cases requiring hospitalisation.

This document reviews biting nuisance and factors that raise the potential for mosquito born disease with the focus on present and near future health risks in urban areas of Britain. Examples of biting and disease transmission by mosquitoes in other temperate countries illustrate the nature of the problem, why it is getting worse and why fears are growing.

Previous reviews, largely prompted by a consideration of global warming or the threat posed by West Nile virus (WNV) and other emerging or re-emerging diseases, provide more comprehensive and in depth coverage. The list of British mosquitoes has been examined and speculation made over potential disease vectors and primary risk factors (Colwell *et al*., 1998;Crook *et al*., 2002;Gratz, 1999;Gratz, 2004b;Gubler, 2002;Higgs *et al*., 2004;Lundstrom, 1999;Medlock *et al*., 2005;Medlock *et al*., 2007).

The intention is not to duplicate or summarise recent reviews and analyses, which should be read in conjunction with this report, but rather to add new perspectives and new data.

Of particular note is the real and present threat to human and animal health resulting from human actions that unintentionally and often unpredictably create or exacerbate mosquito problems (see Box 1).

In a dense urban environment almost every aspect of mosquito survival is affected by human activities, but they do survive and can thrive. London is no exception among large cities in having a long history of localised mosquito biting outbreaks. These vary from temporary, or easily resolved, to recurrent and intractable problems. The difficulties with the latter have been partly due to a lack of general and specialised knowledge on mosquitoes among the public and local authorities and where specialists have been involved a paucity of essential data, but mostly the problems really are difficult to control. A factor is that the mosquitoes often go overlooked until something as simple as a blocked drainage ditch causes a sudden and rapid expansion of the population.

It should not be difficult to see how a construction project on the scale needed for the 2012 Olympic Games, covering an area twice the size of Heathrow's Terminal 5, could easily impact on local mosquito populations. It is one of the biggest urban regeneration project in Europe in over 150 years and will encroach on areas that are home to urban and semi-urban populations of mosquitoes known to bite humans and known to be capable of disease transmission.

The huge influx of visitors to London during the games may not dramatically exceed the numbers normally expected, but the numbers travelling to east London will be unprecedented. Equally unprecedented will be the diversity of countries represented.

At best visitors are likely to encounter biting mosquitoes in relatively small numbers in one or more areas. At worst, the city could see a major biting outbreak, the arrival of people infected with mosquito borne pathogens, the arrival of more dangerous foreign variants of native mosquitoes, or a more dangerous species.

Any or all of these events could drastically raise the risk of a disease outbreak among humans or domestic animals. They can happen at any time and have happened in the recent past.

In this report it is argued that improved knowledge, targeted control measures and monitoring are needed to ensure that current mosquito problems do not escalate and that risks are reduced. The problems are complex and largely unpredictable, so a proactive strategy of education, detection and prevention is better than a misconceived or misplaced, contingency plan.

Box 1: The results of human actions can increase health risks due to mosquitoes

1. **Favourable environments for mosquitoes:**
   - Standing water for aquatic life stages
   - Shelter for overwintering adults
   - Suppression of predators
2. **Favourable environments for reservoir hosts of mosquito borne pathogens:**
   - Provision or removal of a station for migratory birds
   - Maintenance of caged birds
   - Urban habitats for birds and wildlife such as gardens and parks
3. **Passive transportation of mosquitoes**
   - Carrying harmful pathogens leading to infections among humans in transit or living near ports
   - Spreading invasive species
   - Spreading geographical variants of indigenous species that carry undesirable heritable traits
4. **Deliberate or passive transportation of reservoir hosts such as birds or rabbits**
5. **Travel leading to mosquito borne pathogen infection and transfer to a non-endemic country**

2.0 Executive summary

An unprecedented epidemic of West Nile Fever started in New York in1999 and spread across North America in four years. Firm predictions and lessons learned did not prevent a first-time epidemic of Chikungunya in Northern Italy in 2007, illustrating the suddenness with which a mosquito borne disease can appear and the difficulties of planning for such an eventuality.

Britain's last major outbreak ended in 1921. It can happen again and it probably will, but any assessment of risk is hampered by insufficient data and knowledge, many pathogens and vectors, reservoir hosts and myriad diverse factors with complex interactions.

Contingency plans are only as good as the predictions upon which they were based and may not provide sufficient safeguard. Alternatively a comprehensive pre-emptive strike to reduce or eliminate all perceived risks is not feasible, cost effective or guaranteed to succeed.

Nevertheless, a proactive approach that encompasses surveys and selected research activities to underpin development of a fully integrated programme of comprehensive monitoring and targeted mosquito management is needed.

Events in other temperate countries, especially on the European subcontinent, illustrate the nature and underlying causes of a growing threat. Most prominent and generally unpredictable are the factors contributed by humans, especially increased transport and trade and land use changes, which are more immediately relevant than climate change.

At present Britain has about thirty-three species of mosquitoes; mainly human biting and including known or potential vectors of disease. Non-human biting species may also amplify and transmit pathogens, primarily arboviruses, among animals or birds that act as reservoir hosts for human diseases, or are themselves of domestic or economic importance.

Evidence of arboviruses in birds has already been found in Britain and several thousand people infected with mosquito borne pathogens enter the country every year.

Mosquito biting nuisance causing stress and medical problems is present in many locations across britain, including London and other cities. This is a serious health issue requiring effective mosquito control measures, but it is also an obvious focal point in considering the risk of disease transmission.

Culex pipiens molestus causes serious biting nuisance in urban London and other cities in Britain is. It will also bite birds and could transfer an arbovirus like West Nile, from bird to human. It has to be distinguished from the common bird biting biotype *Culex pipiens pipiens* using a DNA based diagnostic test, now available as a service in London. Up to twenty more species, including many biting pests may be found in city gardens, parks and suburban rural areas.

Britain is at risk from invasive species such as *Aedes albopictus*, which so far has been recorded in nine European countries and caused the Chikungunya outbreak in Italy. Passively transported new arrivals have been recorded in surveys of aircraft or inferred from highly localised and temporary disease transmission in the vicinity of ports, such as "airport malaria".

The arrival of foreign variants of native species is less obvious. An example was a single insecticide resistant *Cx.p.molestus* mosquito from southern Europe that founded a population in Scotland resulting in biting nuisance lasting five years.

The components of a mosquito borne disease outbreak and some of the necessary combinations, interactions and circumstances already exist in Britain. Further contributions are on the doorstep. Before now, important mosquito problems required a concerted response that has fallen short and this needs to be redressed to meet a much greater challenge.

3.0 Recommendations

It should be apparent from the above that there is a diverse and complex range of threats to the health of humans and to domestic and economically important animals attributable to mosquitoes. For many aspects of the problem prediction and risk assessment is still little better than speculation and guesswork and so until we are better informed and have better tools at our disposal, contingency plans will provide few safeguards and serve only to promote complacency. On the other hand an assumption that we know enough to implement widespread control is likely to prove expensive both in money and blighted, or lost, lives.

Implementation of the following list of suggestions would initiate early warning and preventative measures dealing with the more immediate and less speculative issues, while moving towards a more informed position. None of the measures need to be prohibitively expensive and could be implemented in stages and/or in different degrees of depth or coverage. Once started the process can generate and put in place meaningful contingencies.

1. In general

- A proactive, but focused and effective approach to reduce mosquito related health hazards and health risks
- Emphasis on data gathering using current knowledge and available techniques to allow informed decision making on appropriate and directed preventive and/or control measures
- Support for research and other measures:
  - to improve knowledge on mosquito related health hazards in Britain
  - to refine risk assessment
  - to enhance data gathering strategies and techniques
- Raise awareness among, and inform, the general public, local authorities and health and environmental agencies

2. To the Olympic committee

- A survey of mosquito breeding in the vicinity of Olympic sites at stages during development
- A review and risk assessment of the final Olympic site plans in relation to known mosquito populations in neighbouring areas
- A review and risk assessment of the final Olympic site plans in relation to reservoir hosts for mosquito borne pathogens
- A review and risk assessment of the numbers and origin of people likely to visit the Olympics to determine if monitoring for mosquito borne infections is necessary

3. To relevant London authorities

- An extension of mosquito surveys in semi-urban areas of London
- A representative survey of mosquitoes across urban London primarily to:
  - identify major *Cx.p.molestus* overwintering sites
  - determine the extent of overground breeding of *Cx.p.molestus* in summer
  - determine the isolation of *Cx.p.molestus* populations
  - confirm the absence of hybrids
  - confirm and measure the extent of host biting preferences
- Development of an integrated London wide control and monitoring programme for *Cx.p.molestus* and other mosquitoes that bite people
- An extension of lessons learned from London to other British cities
- Development of an integrated monitoring programme across Britain to screen for
  - Invasive mosquito species
  - Potentially harmful foreign variants of common indigenous mosquito species
  - Viral infections in reservoir hosts
  - Viral infections in mosquitoes
  - Surges in mosquito populations and associated biting outbreaks
- Development and evaluation of mosquito control strategies
- Encourage an expansion of public education on mosquitoes and associated health risks
- Encourage an expansion of amateur and professional participation in MosquitoWatch and an expansion of identification services and data analysis

4.0 Public health implications

1. Biting nuisance and allergic responses

Severe mosquito biting nuisance occurs across large areas of Europe wherever suitable conditions prevail, including seemingly unfavourable northern regions and dense urban environments.

In general, mosquito problems are spatially and temporally more extensive in warmer climates and control programmes are larger and more established. There is better local public awareness and education regarding self defence against biting and prevention of mosquito breeding. There continues to be a heavy dependence on insecticide, with the consequence that various insecticide resistance mechanisms are now present in high frequencies in the target mosquito populations.

The problems elsewhere are usually more localised and short-lived, but still important and potentially more so, because of poor preparedness and no infrastructure to implement control.

British visitors to Scandinavia may be surprised to find that many areas, mainly in the north, are badly affected by mosquito biting nuisance from June to September and have suffered disease outbreaks. Sweden, most notably, has 48 species of mosquito, the majority of which bite mammals (Schafer & Lundstrom, 2001).

Perhaps even more surprising is the fact that large populations of human biting adult female mosquitoes can be found in midwinter in parts of London and in other major European cities.

Incessant biting alone presents a health hazard that ranges from loss of sleep and severe stress to serious infection. Less well known, and often unrecognised, are the cases of severe allergic reactions to bites (called “skeeter syndrome” in Canada) that can occur in young children, immune deficient people and those previously unexposed to mosquitoes.

Mosquito bites contain allergenic polypeptides from the mosquito saliva, which will cause localised allergic reactions forming pruritic weals and later very itchy bite papules that may last several days.

More serious arthus-type local and systemic symptoms also occur, but anaphylaxis, a severe whole-body allergic reaction, is rare. It is more likely that a bite will lead to cellulitis, an acute inflammation of skin connective tissue caused by bacterial infection (*Staphylococcus, Streptococcus*) and it is usually this that can lead to hospitalisation.

However it is possible for non-infected bites to cause large local inflammatory reactions accompanied by fever in especially sensitised individuals, which can be misdiagnosed as cellulitis and lead to inappropriate treatment.

As biting nuisance spreads to new areas the problem is exacerbated, because previously unexposed people are more likely to show a high level of allergic reaction and will be unfamiliar with procedures for diagnosis, treatment and prevention. The same argument applies to visitors going to temperate areas where there is mosquito problem, particularly if they arrive unprepared. It is often the overall impact on tourism that is the major driving force behind control efforts.

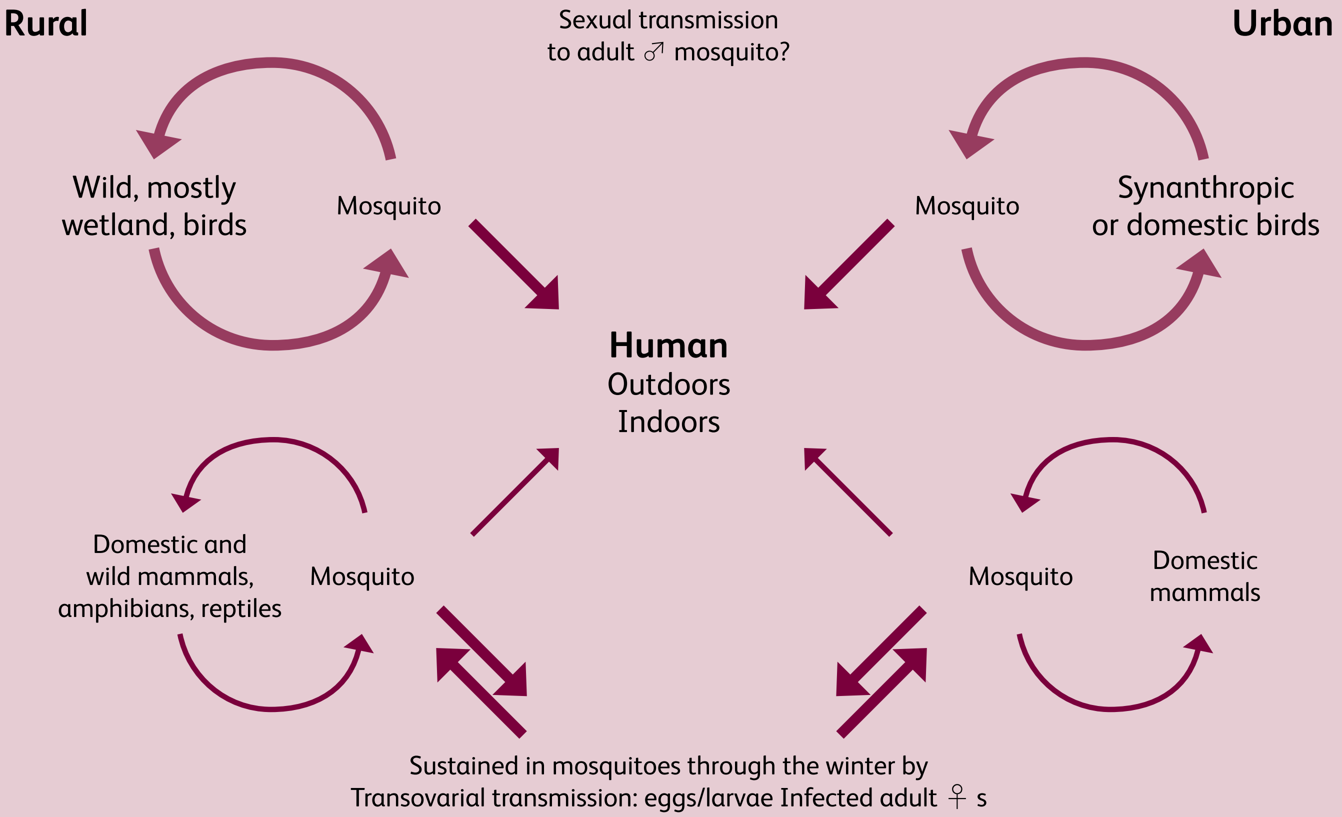
2. Emerging and re-emerging mosquito borne diseases

Globally, human and animal disease outbreaks due to mosquito borne pathogens are becoming more frequent and the number of pathogens involved is increasing.

In temperate countries arthropod borne viruses (arboviruses) pose the most immediate threat and the most intractable problem; of over 550 known, about a fifth cause human disease (Gould *et al.*, 2006;Gratz, 2004b).

Figure 1

West Nile virus: dynamics



The status of disease incidence and risk in Europe has been covered in several detailed reviews (Dauphin *et al.*, 2004;Gratz, 1999;Gratz, 2004b;Gubler, 2002;Hubalek, 2000;Lundstrom, 1999) and recently with particular reference to Britain (Gould *et al.*, 2006;Higgs *et al.*, 2004;Medlock *et al.*, 2007;Medlock *et al.*, 2005).

The evidence ranges from disease outbreaks to surveys based on virus isolates and serology among humans, animals, birds and mosquitoes. The best known, and also the most important mosquito borne pathogens affecting humans after malaria, are the four serotypes that cause dengue and dengue haemorrhagic fever.

There have been no major outbreaks in Europe since the last one in Greece in 1927-28. The main vector, *Aedes aegypti*, is no longer present, but an alternative vector, *Aedes albopictus*, of growing significance globally, is well established in Italy and is spreading (Gratz, 2004b).

In contrast to many other arboviruses travellers infected with dengue can be the primary source for a new outbreak.

At present in Europe, Tahyna virus, Batai (Calovo) virus (Bunyaviridae) and Sindbis (or Sindbis-like) virus (Togoviridae), are the most widely distributed, extending from Russia and Scandinavia to the Mediterranean. West Nile virus (WNV) (Flaviviridae) occurs across central and southern Europe, whereas Inkoo virus (Bunyaviridae) has only been recorded in the north. Others that so far have only been recorded in two or three countries are Lednice virus (Bunyaviridae), a Semliki Forest complex virus (Togaviridae) and Usutu virus.

These are all zoonotic viruses that are amplified in wild birds - the reservoir host - and then transmitted to mammals. The viremia levels are normally only high enough in birds to infect mosquitoes, so a critical factor in determining disease risk among humans or domestic animals is the presence of a bridge vector: a mosquito that will bite birds and mammals (Figure 1).

Serological studies on resident and migratory birds suggest that a Sindbis-like virus known as Ockelbo in Sweden, Tahyna virus, WNV and Usutu virus are circulating among birds in Britain. Tahyna virus and Ockelbo virus cause debilitating, but non-fatal illnesses in humans and are clearly not limited by cold climates.

Tahyna virus poses the greatest risk as rabbits will also act as a reservoir host and so transmission to humans is less limited by the need for a bridge vector (Buckley *et al*., 2003;Buckley *et al*., 2006;Gould *et al*., 2006).

WNV attracted a lot of attention in Britain following its sudden appearance in the USA in 1999, where over 27618 cases resulting in1086 deaths have since been reported.

WNV causes West Nile fever and when it extends to the nervous system the more severe forms of the disease: West Nile encephalitis, an inflammation of the brain, and West Nile meningitis, an inflammation of the membrane around the brain and the spinal cord.

Most transmission of the virus occurs among wild birds vectored by bird biting mosquitoes. These cycles can be distinctly rural or urban. Frogs have been shown to be a possible source of infection for mosquitoes, but mammals do not usually produce sufficient viremia to maintain a transmission cycle.

However, mosquitoes regarded as non-bird biting are found with WNV infections, so there is some limited transmission with non-avian vertebrate hosts and/or these mosquitoes are biting birds (Hayes *et al*., 2005; Hubalek, 2000).

In 2002, the Chief Medical Officer (CMO) for England identified the major factors contributing to the risk of WNV transmission to humans as: potential vectors within the existing mosquito population, WNV infection in the domestic bird population and global warming.

The first two factors are obvious and again emphasise the need for more basic data and monitoring. The third, global warming, overshadows more immediate and more directly relevant aspects of the problem such as:

- The significance of the seasonal factors that bring the maximum congregation of migratory birds together with the expansion of the mosquito population (Hubalek, 2004);
- A consideration of the full range of ongoing changes in the environment affecting both birds and mosquitoes, eg. land use, pesticides, building projects
- The introduction of foreign mosquitoes; either invasive species or variants of indigenous species assisted by increasing national and international transport and trade
- Genetic changes in arboviruses, which occur with a high frequency and can result in dramatic changes in transmission dynamics and the clinical consequences of infection

The WNV that appeared in the USA is actually an example of the last point (Blitvich, 2008). Others include Ockelbo virus in Sweden, which is probably a locally evolved and more dangerous variant of Sindbis virus (Gould *et al*., 2006), and the recent widespread outbreak of a variant of Chikungunya virus (Tsetsarkin *et al*., 2007;de Lamballerie *et al*., 2008)

The inadequacy of the CMO's assessment illustrates the often conflicting views offered by experts and in turn the near impossible task of providing good estimates of disease risk, or even to rank the factors involved with any degree of confidence.

This is exemplified by the various explanations given for the unprecedented appearance of WNV in New York in 1999 and its rapid spread throughout the USA and parts of Canada within four years. It is an issue because it contrasts markedly with the outbreaks in southern and central Europe which have been sporadic, short lived and fairly isolated.

Despite an exponential rise in scientific publications on the topic over the last decade, there is still a poor understanding of the underlying causes. A singular problem is the identification of mosquito vectors, as many species have been implicated and the primary vector, *Culex pipiens sensu lato*, is a poorly resolved species complex.

Chikungunya, another emerging disease, is more straightforward. Yet despite a raised level of awareness and apparently firm high risk predictions, a first-time disease outbreak in Europe was not prevented (Queyriaux *et al*., 2008).

Chikungunya is an alphavirus of the family Togaviridae transmitted by mosquitoes, primarily *Ae. aegypti*, but of more relevance here is *Ae. albopictus*, which has recently come to the fore.

Chikungunya is generally considered a tropical non-fatal disease that causes excruciating pain in the wrists and ankles lasting a month or so, but a more severe and sometimes fatal clinical form has been recorded in significant numbers.

A series of major disease outbreaks caused by a new variant of the virus started in Kenya in 2004 and spread through islands in the Indian Ocean, in particular Reunion, to reach India by 2006 (Charrel *et al*., 2007).

An outbreak then occurred in two villages in north eastern Italy in 2007, thought to have been initiated by a visitor from India, who only developed symptoms after his arrival (Rezza *et al*., 2007). The new form of the virus appears to be more adapted to *Ae. albopictus*, which previously was not considered to be a primary vector for Chikungunya, and far more harmful (Tsetsarkin *et al*., 2007;de Lamballerie *et al*., 2008).

In contrast to WNV, mosquitoes can transmit the virus from human to human so the the dynamics are less complex. This illustrates the important role of international travel in the spread of dangerous pathogens. The outbreak in Italy arose because an invasive species, *Ae. albopictus*, was introduced through the used tyre trade and then the pathogen was introduced by a visitor.

Measures that can be put into place to reduce risk from a disease like Chikungunya include monitoring for exotic mosquito species and travellers infected with pathogens (Queyriaux *et al*., 2008).

In contrast, infected travellers do not pose a risk for diseases caused by WNV and other viruses with a reservoir host and where mammals are a "dead-end host". Instead the reservoir host has to be monitored. Exotic mosquito species are still an issue, but as will be illustrated monitoring for foreign variants of indigenous species is equally important.

5.0 Factors influencing public health

1. Geographical variants and biotypes of mosquitoes

Mosquitoes that belong to the same species may be identical in appearance and vary in their ability to interbreed, yet still show a wide range of distinct characteristics that are relevant to biting nuisance, disease transmission and control. These variants may be associated with their place of origin, a particular ecological niche or a specific biotype. An example is *Cx. pipiens* s.l., the most abundant and widespread mosquito in the world.

Cx. pipiens s.l. is a complex of morphologically indistinguishable, physiological, behavioural and geographical variants of uncertain taxonomic status.

In Britain there are two distinct biotypes that differ in their biting and mating behaviour and in egg production (Box 2.) (Chevillon *et al*., 1998;Harbach *et al*., 1985;Marshall & Staley, 1937;Marshall, 1944;Roubaud, 1933;Vinogradova, 2000).

In southern Europe the two forms can interbreed. Cold winters may eliminate hybrids, but do not entirely counter gene flow, particularly of selected traits such as insecticide resistance, and can result in mixing of characters normally specific to each form (Chevillon *et al*., 1995;Pasteur *et al*., 1977).

Hybrids or intermediate forms are more likely to be good bridge vectors for arboviruses such as WNV, picking up the virus from birds and transmitting it to humans. This could provide part of the explanation for why WNV epidemics in Europe have been localised and short lived.

A more extreme situation exists in the USA, where a novel form that resembles a hybrid between forms *Cx. p. molestus* and *Cx. p. pipiens* has been found. Its prevalence and ability to survive the winter is thought to have augmented the severity and rapid spread of the human West Nile fever epidemic (Fonseca *et al*., 2004).

On the other hand, the relative isolation of typical *Cx.p.molestus* breeding sites in Europe can result in significant differences between neighbouring populations (Chevillon *et al*., 1995).

Also, mosquitoes infected with different strains of *Wolbachia* (endosymbiotic bacteria that infect a wide range of arthropods transmitted via the cytoplasm of the egg) may cause reproductive problems in certain crosses, further restricting gene flow between populations, irrespective of form (Duron *et al*., 2005;Magnin *et al*., 1987).

In Egypt and most of Israel, there is really only one form, the populations are steganomous and primarily mammophilic, but variable for autogeny (Gad *et al*., 1995;Nudelman *et al*., 1988). The tropical form, *Cx. quinquefasciatus*, is often considered a separate species. It is reproductively isolated from temperate *Cx. pipiens* in South Africa, but not in California (Cornel *et al*., 2003).

All of the above illustrates just some of the complexities that have to be dealt with for species like these. It is relevant to Britain because:

- The two native biotypes show important differences compared to their continental counterparts relevant to biting nuisance, disease transmission and control
- Any of the above variants could find their way to Britain and will be largely indistinguishable from native forms. Some already have

2. Mosquito migration and dispersal

Most mosquitoes do not travel long distances of their own accord and migration is normally gradual (Service, 1997). Changes in the environment may support or counter migration and so help to expand or contract the geographical range of a species, but not necessarily in a dramatic way, even with the current evidence for global warming.

Box 2. British *Culex pipiens s.l.*

Cx. pipiens pipiens,
- Common and widespread
- Bird biting (ornithophilic),
- Requires blood for egg production (anautogenous)
- Open space for mating (eurygamous)
- Breeds overground (epigeous)
- Hibernates (heterodynamic) in winter

Cx. pipiens molestus
- Mostly urban isolated populations
- Bites mammals (mammophilic)
- Can produce eggs without blood (autogenous)
- Does not hibernate
- Passes the winter in underground (hypogeous) or sheltered constant temperature sites
- Mates in confined spaces (stenogamous)

Mosquitoes can be dispersed by other means such as wind. Normally this would not be on the scale of smaller insects like midges, which were carried from the continent to south east England and caused the recent outbreak of blue tongue virus (IAH, 2007), but there is evidence for the introduction of Japanese encephalitis into Australia from New Guinea by wind-borne mosquitoes (Chapman *et al*., 2003;Johansen *et al*., 2003;Ritchie & Rochester, 2001).

Human transportation and trade is more important. Mosquitoes can be passively carried around the globe, because they are temporarily trapped in vehicles, or cargo containers, or even living in the cargo, for example in used tyres (Lounibos, 2002;Tatem *et al*., 2006a).

"Airport malaria"
There are many examples of mosquitoes found in transit. Presumably these are a tiny fraction of the actual number of events since they only come to light when specific surveys are made.

Most will die without consequence.
For example a survey in Britain undertaken in 1983 found tropical disease vectors, including *Cx. quinquefasciatus*, arriving on aircraft (Curtis & White, 1984).

If the mosquitoes are infected and survive long enough they may bite travellers, or residents living near ports. The temporary presence of the mosquito is implied from cases of a mosquito borne disease among people who have never been to an area with a risk of infection.

There are many records of clusters of mosquito borne disease cases near ports, in particular "airport malaria", which have occurred repeatedly in western European countries, including Britain (Baixench *et al*., 1998;Cimerman *et al*., 1997;Gad *et al*., 1995;Giacomini, 1998;Gratz *et al*., 2000;Guillet *et al*., 1998;Isaacson & Frean, 2001;Jafari *et al*., 2002;Karch *et al*., 2001;Lounibos, 2002;Lusina *et al*., 2000;Marty *et al*., 2001;Mouchet, 2000;Praetorius *et al*., 1999;Rabinowitz *et al*., 2004;Tatem *et al*., 2006b;Tatem *et al*., 2006a;Thang *et al*., 2002;Van den *et al*., 1998;Warhurst *et al*., 1984;Whitfield *et al*., 1984)

Short stay foreign relatives
If foreign relatives of an indigenous population arrive and survive long enough to mate with the local mosquitoes, the event is highly unlikely to be detected. As with "airport malaria" it is inferred from the consequences.

Box 3. Mosquitoes: New arrivals

"Airport malaria"
- Typifies the worst case scenario
- Mosquito arrives, bites and dies
- Infected mosquitoes survive long enough to bite people in transit or living near ports

Short stay foreign relatives
- Arrive, mate with locals and die.
- In worst case pass on undesirable traits to local mosquitoes
- Typified by the spread of insecticide resistance

Long stay foreign relatives
- Arrive and survive
- May or may not introgress with the local population
- Worst case is a dangerous variant

Invasive species
- Arrive and survive
- Worst case is a dangerous vector

An illustration is provided by *Cx. pipiens* s.l. where very long range dispersal of different variants is indicated by the rapid spread of known insecticide resistance alleles.

Most dramatic is the A2B2 haplotype, comprising two co-amplified esterase genes, which confer organophosphate insecticide resistance. This haplotype evolved in African *Cx. quinquefasciatus*, but within decades was found in other *Cx. pipiens* forms and around the world (Labbe *et al*., 2005;Labbe *et al*., 2007).

Insecticide used in control operations selected for the introduced resistance and countered the fitness cost of the amplified genes (Bourguet *et al*., 2004). A detailed study in southern France monitored the spread of A2B2 from its point of arrival in Marseilles and the gradual replacement of the resistance alleles that had evolved locally.

The undesirable trait spread through the local population following the arrival of a foreign variant in Marseilles and not foreign variants themselves, or their direct descendents. It would not have spread so rapidly if organophosphate insecticide had not been in use, so the current known distribution of A2B2 is largely congruent with control efforts to combat disease transmission or serious biting nuisance.

Long stay foreign relatives
Ordinarily, A2B2, or other similar elevated esterases conferring organophosphate resistance would not be expected in Britain, however A2B2 was found in an isolated population of *Cx. p. molestus* in a yeast factory in Scotland in 2001. These mosquitoes had been causing serious biting nuisance and looked at first to be a typical chance infestation of business premises not too dissimilar to the only previous record in Scotland, less than 50 km to the south in Edinburgh (Ramsdale & Snow, 1995). After a detailed biochemical and molecular analysis the only plausible evidence for the presence of the population was an example of the fourth scenario listed in Box 3.

A single inseminated female carrying the A2B2 haplotype was transported, probably by container lorry, from somewhere in southern Europe. In the yeast factory it found an ideal site to lay its eggs in perpetually flooded subterranean channels built for high voltage cables. The cables provided extra warmth and the yeast extra food.

The population spread across the factory and survived with little disturbance until eradicated by drainage of the breeding sites and treatment with Bti insecticide. With the exception of a small housing estate where most of the biting was taking place, the factory was surrounded by farmland which limited the scope for the mosquito to spread further.

The farms were all organic and no insecticide had been used in the area previously either against the mosquitoes or for any other purpose. The high frequency of the A2B2 haplotype was therefore sustained by the lack of any opportunity to out-breed with local mosquitoes.

If the event had taken place in Edinburgh or any other city, there is a high likelihood that outbreeding would have occurred. This could have led to the spread of insecticide resistance or other undesirable traits into the indigenous population.

Were it not for the insecticide resistance the translocation event described above would have been entirely undetected.

Invasive species
More obvious are invasive species. Mosquitoes previously unknown in an area arrive and not only survive, but succeed in founding a population.

The most serious example was the translocation of the African malaria vector *Anopheles gambiae* to Brazil in 1930, which resulted in 16,000 deaths (Soper & Wilson, 1943), but more recent and much more widespread is *Ae. albopictus*, the tiger mosquito, which has been highlighted as one to watch out for in Britain (Ramsdale & Snow, 2000).

Based mostly on laboratory studies it has been shown to be a competent vector for at least 22 arboviruses. It is a native of South-East Asia, but within the last three decades has been found in Africa, Israel, Europe and North and South America.

The eggs of this species can survive months of desiccation, which has facilitated its dispersal via the international trade in used tyres (Charrel *et al*., 2007;Gratz, 2004a;Medlock *et al*., 2006). In fact the intercontinental spread of this species has been shown to be predictable based on high shipping frequencies among ports with comparable climates.

The records of *Ae.albopictus* breeding in Europe started with Albania in 1979 and Northern Italy in 1990 and now extend to ten countries including Spain, France, Belgium and the Netherlands. It is the most serious biting pest in Italy and as already stated above was responsible for the recent outbreak of Chikungunya (Rezza *et al*. 2007).

Medlock *et al*. (2006) have evaluated the prospects of *Ae. albopictus* surviving in Britain. They note there is a high risk of it arriving given the importation of in excess of five million used tyres per year. They conclude that *Ae. albopictus* could establish itself across much of lowland Britain including urban areas in and around London, where it would cause a biting nuisance and disease threat throughout the summer.

3. Changes in the environment

The debate over global warming and its consequences has moved from ardent denial to widespread acceptance, with many predictions of associated health hazards including an expansion of mosquito borne disease.

Climate change is however only one component of a complex set of factors that are all contributing to the growing risks posed by mosquitoes. Global warming could support the expansion of a mosquito vector by migration, but it could also enable a far more dramatic expansion by aiding the survival of a passively transported mosquito.

It could affect the distribution of non-human hosts that act as a reservoir for the disease pathogens transmitted by mosquitoes. More favourable climatic conditions will also assist the amplification and spread of pathogens following introduction through human and animal travel.

Global warming will therefore facilitate and exacerbate mosquito biting nuisance and disease transmission, but many other factors are arguably more important and more immediate.

One factor that is now receiving more attention is change in land use. It is self evident that a local change in the environment can directly affect mosquito populations, their hosts and the disease pathogens they transmit, but not necessarily all at once or in a way that is sustainable.

The consequences of a national trend may be less conspicuous, but can lead to a more long-term impact and importantly bring about multiple changes, for example bringing together reservoir hosts and competent vectors.

This can be complex as illustrated by three studies on WNV.

The first examined two areas in southern France, the Camargue and Var, which are both endemic for WNV, but ecologically quite different. The heterogeneity of landscape characteristics was found to be important in promoting the co-existence of the mosquitoes and reservoir hosts and it was this that the two locations had most in common (Pradier *et al*., 2008).

In the USA, one study in mid-Atlantic region found that seroprevalence in wild mammals increased along a gradient from forest to urban land use (Gomez *et al*., 2008) whereas a study in Iowa, found that WNV diseases in humans was correlated to rural farmland (DeGroote *et al*., 2008). In each case land use was found to be highly significant, but of course not the only factor. A recent and more obvious example is the resurgence of Anopheles in Italy following an expansion of rice cultivation.

It is difficult to predict, where and how changes in land use will increase mosquito borne disease risk, a simpler task is to analyse if land use was a factor in the elimination of a disease.

In considering the risk of malaria returning to Britain several studies have asked why malaria disappeared in the first place, climate was considered to play apart, but overall changes in use of land and agricultural practices were prominent factors alongside improved health care and living standards (Dobson, 1994;Hutchinson & Lindsay, 2006;Kuhn *et al*., 2003;Lindsay & Joyce, 2008;Reiter, 2000).

A firm prediction is that the distribution and abundance of wildlife will change with changes in land use and there are identifiable trends in Europe, which include a decrease in agricultural land, increases in forestry and nature reserves, more road building and urbanisation.

Included within this may be significant underlying trends, for example fewer or smaller gardens in city centres with the increase in apartment blocks, more structured nature reserves allowing greater public access to get “closer to nature”, perhaps more or larger parks, but less formal, in towns and cities and an increase in the use of rivers for both transportation and leisure.

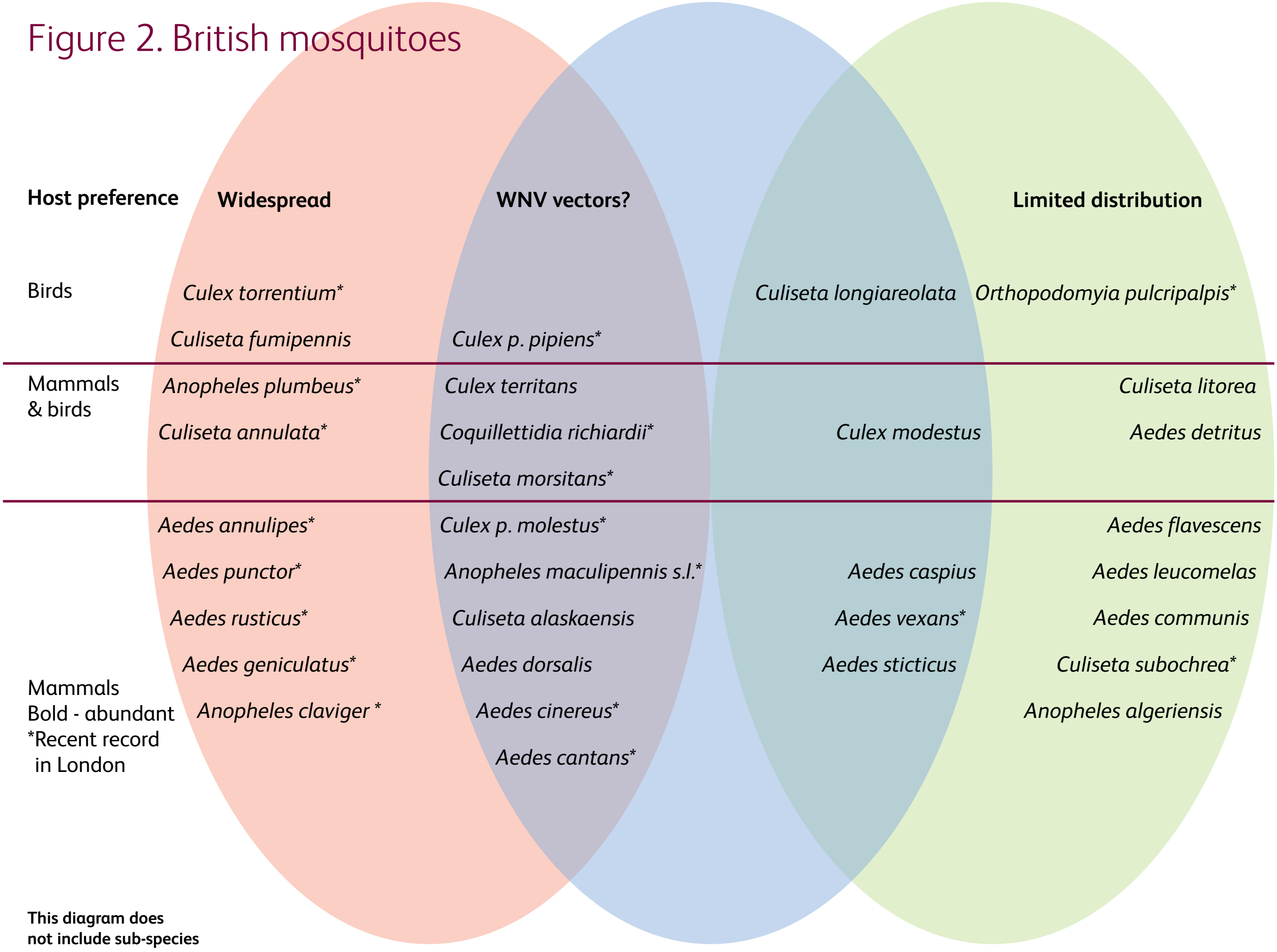


Figure 2. British mosquitoes

Host preference	Widespread	WNV vectors?		Limited distribution
Birds	*Culex torrentium** *Culiseta fumipennis*	*Culex p. pipiens**	*Culiseta longiareolata*	*Orthopodomyia pulcripalpis**
Mammals & birds	*Anopheles plumbeus** *Culiseta annulata**	*Culex territans* *Coquillettidia richiardii** *Culiseta morsitans**	*Culex modestus*	*Culiseta litorea* *Aedes detritus*
Mammals	*Aedes annulipes** *Aedes punctor** *Aedes rusticus** *Aedes geniculatus** *Anopheles claviger* *	*Culex p. molestus** *Anopheles maculipennis s.l.** *Culiseta alaskaensis* *Aedes dorsalis* *Aedes cinereus** *Aedes cantans**	*Aedes caspius* *Aedes vexans** *Aedes sticticus*	*Aedes flavescens* *Aedes leucomelas* *Aedes communis* *Culiseta subochrea** *Anopheles algeriensis*

Bold - abundant
*Recent record in London

This diagram does not include sub-species

6.0 British mosquitoes

1. Mosquitoes across Britain

Thirty three species of mosquito have been recorded in Britain, these are shown in Figure 2, which provides a simplified categorisation based on distribution and host biting preference.

The scope of this document does not cover a detailed analysis of potential vectors for the full range of mosquito borne pathogens; instead WNV is used to illustrate some important considerations that are general, or of specific relevance to arboviruses with an avian reservoir host. Consequently, species for which isolations of WNV have been recorded are also indicated in Figure 2.

Species without such records were probably not studied and should not be excluded from consideration, especially since over 100 species have now been shown to be susceptible to infection and even some that were fairly refractory were still found to be capable of transmission (Hubalek & Halouzka, 1999;CDC, 2007). A more detailed consideration of potential arbovirus vectors can be found in (Medlock *et al*., 2007;Medlock *et al*., 2005).

Nearly all speculation over the capacity of British mosquito species to transmit pathogens is derived from data in other countries. This sort of extrapolation must be treated with caution, since different populations of the same species can show markedly different characteristics.

An example is British *Cx.p.pipiens*, which is infected with a *Wolbachia* strain that is different to any found in *Cx.pipiens s.l.* on the continent. This confirms the isolation expected of an island population and, due to the mating incompatiblities conferred by *Wolbachia*, provides an additional reproductive barrier. It is therefore quite feasible that British *Cx.p.pipiens* is dissimilar to relatives abroad in many other ways, including vector competence.

Another issue is identification. Although the two representatives of *Cx. pipiens* s.l. in Britain are not recognised as separate species aspects of their biology relevant to biting nuisance, disease transmission and control are so different (Box 2) that they must be distinguished.

Another complex is *An. maculipennis* s.l., which is represented in Britain by two morphologically indistinguishable species, *An. atroparvus* and *An. messeae*. Both species are vectors of malaria.

An. atroparvus was responsible for the last major outbreak from 1917 to 1921 in the vicinity of the Medway estuary, Northern Kent and is regarded as the more efficient vector. *An. messeae* was however implicated as the major cause of malaria returning in the Ukraine and Russia (Nikolaeva, 1996;Bezzhonova & Goryacheva, 2008). The two species favour different larval and overwintering habitats, but can be found in sympatry.

An. daciae is a third member of the complex that may also be present. It was given species status (Nicolescu *et al*., 2004) , but this has not been supported by recent data (Bezzhonova & Goryacheva, 2008).

Accurate population studies are therefore only possible with good identification methods. These became available relatively recently, but involve a moderate cost because the diagnostic assays are based on PCR of DNA.

In fact even without these tools few detailed studies of mosquito population dynamics have been made. It is therefore surprising that any reliance has been placed on an often quoted comment that the risk of WNV transmission in the United Kingdom is remote, because the population density of potential vectors is too low (Crook *et al*., 2002). It is at best an oversimplification; population density is variable across the country and it is not especially difficult to find high densities of certain species in the summer, but more importantly mosquito numbers can increase very dramatically in a short time given the right conditions. The latter is often seen associated with new outbreaks of biting nuisance and in fact most studies of British mosquitoes resulted from such events. It is reasonable to assume that while a few cases of West Nile fever or other mosquito borne disease might occur anywhere, a serious outbreak will coincide with a high level of biting.

It is important that non-human biting mosquitoes are also studied and monitored as they may contribute to the pool of pathogens available for human infection, but it is clear that the major priority should be to monitor and tackle mosquito biting nuisance.

There is enough justification on health problems associated with bites alone to demand that local authorities take the problem seriously. Yet many pest control departments don't even have a code for mosquito biting complaints and action depends on how vociferous the complainants are.

The public don't always complain for a variety of reasons, but often it is simply that they are unaware that they can. Unfortunately even when they do, there is a risk that the complaint is not recorded or taken seriously. One environmental health officer discovered that the receptionist was dealing with mosquito biting complaints herself by providing advice on self-defence based on her holiday experiences.

An extensive review of examples of biting problems in Britain is provided by (Ramsdale & Snow, 1995); some are permanent and associated with natural breeding sites, for example *Ae.detritus* on coastal marshlands and others reoccur. The latter may emanate from a permanent primary source as with problems in areas of London, which will be described below.

There are examples of temporary problems that may last up to a few years, but once the underlying cause is established they are relatively easily resolved (where there is a will to do so).

The five year biting outbreak by *Cx.p.molestus* in Scotland described above is one example, where the transportation of the mosquito and the larval habitats were provided by humans and where poor knowledge resulted in denial, intransigence and inaction.

New problems occur every year and not all are investigated, partly because of a lack of infrastructure or acknowledged responsibility to deal with mosquito problems and partly a failure to recognise the problem for what it is.

In 2005, a concerted effort to find new cases of mosquito biting nuisance resulted in no responses, or zero returns, from over 500 local authorities. Nevertheless by other means new problems were identified in Calendar and Grangemouth in Scotland, in small villages in Kent and Nottinghamshire and in areas near Southampton and Lowestoft.

The example in Nottinghamshire serves to illustrate several points. The problem started after three lagoons were created to deal with an area prone to flooding. Ordinarily the flooding might have been expected to be the source of a mosquito problem and the lagoons a more easily managed alternative.

Nevertheless, immediately following the change in the landscape the mosquito population exploded, resulting in clouds of adults rising from any disturbed vegetation anywhere within a kilometre of the lagoons.

It contained seven different species, mostly human biting. It is probable that the habitat change suppressed, or eliminated a predator. Since the species were mostly of the genus *Aedes*, the peak in the incidence of biting occurred when there were no longer many larvae around.

This meant that a full investigation to establish the cause of the problem and to provide a control strategy would have taken at least a year. The use of insecticides would have had an unacceptable impact on local fauna in particular a rare damsel fly.

The affect on local residents was severe, especially children. The local council was not prepared to take steps to investigate, or remedy the problem, because the cost was felt to be disproportionate to the number of people affected and there was no guarantee it would reoccur. It did reoccur and remained a problem, albeit diminished.

In this case the mosquito densities were very high, but localized. It is probable that a number of people will have reacted badly to the bites, but will have become desensitized in subsequent years.

In general there is a tendency to get used to the problem, helped by the use of repellents, wearing more clothing, avoiding the areas where the mosquitoes are resting and other defensive measures. Complaints would then tend to fall in number and the likelihood that local authorities do something about the high mosquito numbers becomes less, but the scope for a disease outbreak remains.

The involvement of seven species increases the likelihood of at least one being a good disease vector. In fact five were among the nearly one third of mosquito species recorded in Britain that have been found infected with WNV elsewhere (Figure 2).

Another feature of the above example is that it would probably have been overlooked by models mapping risk areas. Firstly, because the area involved was so small and secondly because it would not have scored highly on risk factors; it is not realistic to include parameters in a model to account for arbitrary environmental changes with unpredictable consequences. What was more predictable was that these mosquitoes were already there before the population expansion.

Mosquito distribution and biting patterns are the best and the most easily updated data available for judging the risk of biting nuisance and a preliminary assessment of disease risk.

For example, it is true that mosquito population densities are not high across the country for any species and that almost half the number of species have a quite limited distribution. This may change in a year with unusual weather, or long term with global warming, but it can be argued that the risk of a countrywide epidemic of WNV diseases similar to the USA is minimal.

Furthermore most of the widespread mosquito species are primarily mammal biting and will almost certainly play no significant role in amplifying West Nile virus among birds or transmitting the virus to humans. The British mosquito species that are both widespread and bird biting are *Cx.p.pipiens*, *Cx torrentium* and *Cs. morsitans* in rural and semi-urban areas, with perhaps only *Cx.p.pipiens* in fully urban areas.

WNV isolates have been obtained from *Cs. morsitans* and *Cx.p.pipiens* abroad, so these two must be considered good candidates for a significant role in the circulation of WNV. *Cs. morsitans* will also bite humans and so is a potential bridge vector. *Cx.p.pipiens* is much more abundant and can be found breeding in large numbers even in very built-up urban areas. It is however not a likely bridge vector.

The literature is confusing on this, because a number of articles have used data from abroad, or where the biotype was not known. Recent data from studies on translocated insecticide resistant *Cx.p.molestus* in Scotland (see above) and on a native *Cx.p.molestus* population in East London (see below) have shown that the two forms are reproductively isolated and very different in their host biting preferences.

Eight of the ten species most likely to bite humans are predominantly found in rural habitats and are not considered to bite birds, including *Ae.detritus* against which most current control is directed. Apart from *Cx.p.molestus* the remaining major contributor to biting nuisance is *Cs.annulata*. This species is common throughout Britain, will definitely bite birds and can be found in both rural and urban habitats. Despite being found across most of Europe it has not been implicated in WNV transmission, but must be considered a risk. Of the remaining common species *Cq. richiardii*, *An. claviger*, *An. maculipennis* s.l. and *An. plumbeus* merit attention; although not responsible for any serious biting nuisance they will readily bite humans. *Cq. richiardii* and *An. plumbeus* also bite birds; the former has been linked with WNV in Europe.

An. plumbeus breeds in treeholes and can be found in urban gardens. It has been implicated in malaria transmission and may have been responsible for the last outbreak of autochthonous transmission of malaria. *An. maculipennis s.l.* is a species complex represented in Britain by *An. atroparvus* and *An. messiae* with concern.*Ae. vexans* is not common, but has been implicated in biting outbreaks in or around London and was present in low numbers among the species found in the biting outbreak in Nottinghamshire. It has been recorded abroad infected with WNV and if it increases in number it is likely to become a serious biting nuisance, including urban areas (Ramsdale and Snow 1995).

Other considerations are the possibility of enhanced spread of the virus through the mosquito population by sexual transmission and the maintenance of the virus during the winter.

Sexual transmission of arboviruses in mosquitoes is well established, especially dengue, but not yet for WNV. Overwintering adult females of *Cx. pipiens* have been found infected with WNV and thus this species and those with similar characteristics are most likely to contribute to outbreaks in successive years.

Transovarial transmission of WNV has also been shown in *Cx. pipiens*, which means that newly emerging female adults may cause infection with their first bite and that overwintering eggs and larvae could also be carrying the virus.

In summary:

- In both rural and urban areas countrywide, the best candidate vector for amplification of WNV among birds is *Cx.p.pipiens*, but also *Cx. torrentium* in rural areas if it is shown to be WNV competent
- In rural areas countrywide, a second candidate vector for amplification of WNV among birds and as a bridge vector for transmission to humans is *Cs. morsitans*, but also *Cs. annulata* in rural and urban areas if it is shown to be WNV competent
- Other potential bridge vectors, for example *Ae. cantans* require further investigation
- An important candidate bridge vector in urban areas is *Cx.p.molestus* and even more so hybrids between *Cx.p.molestus* and *Cx.p.pipiens*. This has now been investigated and is reported below.
- In the southern half of England, especially the south east:
  - There is a greater diversity and abundance of mosquito species and a greater risk of human biting. There is probably an added risk of WNV transmission to humans from *Cq. richiardii* and *An. plumbeus*
  - There are others on the list of man biting species that could contribute to WNV transmission to humans if found to be also bird biting

2. Mosquitoes in urban environments

Rural and semi-urban

London has many large parks, rural and semi-urban areas that provide a haven for wildlife, which includes a diverse range of mosquito species.

Keith Snow and Jolyon Medlock (unpub.) recently completed a detailed survey in East London conducted over a period of one year starting early in 2007. This provides an up to date and comprehensive illustration of the many mosquito species that can be found living within a short distance from dense human populations.

The study involved regular sampling from ten small sites in Epping Forest and one urban site in nearby Stratford. The topography of the main study area is quite mixed ranging from dense woodland to open grassland crossed by busy roads and encroached on, or in places broken up, by residential and other buildings. It is criss-crossed with popular foot and cycle paths, but landscaping and land management is minimal.

Epping Forest is extensive, stretching from the M25 to inside the North Circular, but even in the most rural sections it would be difficult to get as much a kilometre from a road or residential area.

In total 17 mosquito species were recorded (indicated by an asterisk in Figure 2), of which 12 were only found in natural rural habitats. *Culex torrentium*, and *Culiseta subochrea* were also limited to the rural areas, but larvae were found in artificial containers. The remaining three, *Cx. pipiens* s.l., *Cs. annulata* and *An. plumbeus*, were found at all locations including the urban site.

Cx.p.molestus larvae were found in flooded basements of eight residential and commercial buildings where adult females were causing a biting nuisance all year. *Cs. annulata* and *Ae. vexans* were also biting humans and in addition horses.

Five others, *Ae. cinereus, Ae. cantans Ae. annulipes Ae. geniculatus* and *Ae punctor*, were readily attracted to human bait and would certainly have been biting people when encountered, especially at dusk.

Urban

The urban site described in the previous section is typical. The most commonly found mosquitoes in descending order of abundance being: *Cx.p.pipiens*, *Cx.p.molestus*, *Cs.annulata* and *An. plumbeus*.

Cs. annulata will enter houses and gives a particularly painful bite, but numbers are rarely large compared to either *Cx. pipiens* biotype. Most mosquito biting nuisance in the built-up areas of London have exclusively been due to *Cx.p.molestus*.

This biotype was first identified in Britain following the collection of eggs from a water tank on Hayling Island in 1934 and subsequently confirmed as the cause of biting problems in Westminster and Hull.

At the time the authors made a case for distinguishing this biotype as a separate species and to adopt the name molestus based upon the description of a very similar mosquito from Egypt by Forskal in 1775. It is interesting to note however that they also observed significant differences between geographical strains and cited a study in which a successful cross had been made between the two biotypes(Marshall & Staley, 1937).

This ambiguity has persisted ever since in the literature as data became more convoluted. It is only at the present that we are beginning to understand this species complex. Fortunately in Britain we can now be quite certain that there are only two biotypes and they are distinct. Therefore all of the early records of biting nuisance attributed to *Cx.p.molestus* can be considered accurate.

In studies covering 1938 to1941, Marshall (1944) reported widespread biting nuisance due to *Cx.p.molestus* in London, including Battersea, Bermondsey, Chelsea, Deptford, Greenwich, Isleworth, Peckham, Twickenham, Richmond, West Ham and the London Underground.

One of the longest serving *Cx. p. molestus* habitats, and the most extensive, dates back to at least the Second World War. People were bitten when using the London Underground tunnels as air-raid shelters (Marshall, 1944;Shute,1951).

As the temperature never falls below 15 ˚C the mosquitoes can breed throughout the year, but the depth of the tunnels and the extensive area they cover can make larval breeding sites difficult to find and therefore to remove.

A survey largely directed by biting complaints from staff was made in the mid 1990s. Six breeding sites were confirmed as far apart as Liverpool Street and Shepherd's Bush, east to west, and Finsbury Park and Elephant and Castle, north to south (Byrne & Nichols, 1999). Over the years, improvements to the network have reduced the scope for mosquito breeding, but as recently as 2006 mosquitoes were still biting staff at one central London station.

The early records of biting nuisance in Isleworth, Twickenham and Richmond (Marshall, 1944) may have been the first indications of a second major and long standing habitat for *Cx.p.molestus*, the Mogden Sewage Treatment Works in Isleworth, which was built in the early 1930s.

A third one, although only recently identified, is Becton Sewage Treatment Works in Newham. Both are run by Thames Water Utilities Ltd and are among the largest STWs in Europe.

The presence of *Cx.p.molestus* within Mogden STW was confirmed in the 1980s, since then the biting nuisance has provoked increasing unrest among local residents leading to the formation of the Mogden Residents Action Group in 2001.

Shortly afterwards Hounslow Borough Council served a statutory Abatement Notice on Thames Water and then in 2005 a group action claim was issued in the High Court against the company on behalf of 1300 local residents. This is still ongoing.

Three surveys of mosquitoes in the area were commissioned and reported on in 2003 and 2004(Ismay & Schulten, 2003;Ismay & Schulten, 2004a;Ismay & Schulten, 2004b). Mosquitoes were found breeding outside the STW during the summer, which encouraged the belief that the biting problem is exacerbated by the availability of standing water in gardens, allotments, drains etc., therefore shifting at least some of the blame from the STW to residents and local authorities.

In fact it is easy to find mosquitoes breeding in many diverse locations including gardens and allotments all across London, but in almost every case the mosquitoes are primarily *Cx.p.pipiens*, so not human biting. As the two biotypes are so similar it is impossible to say which is present in the larval breeding sites outside the perimeter of Mogden STW without further diagnostic tests.

This was illustrated the following year (2005) when larvae collected from drainage ditches normally subjected to control measures by Hounslow Borough Council were analysed by a DNA based diagnostic test; very few *Cx.p.molestus* were identified among large numbers of *Cx.p.pipiens*.

This result is entirely consistent with more extensive studies made in the vicinity of Beckton STW (see below), with the London Underground study(Byrne & Nichols, 1999) and historical studies in London. For example, Shute (1943,1949) described Thames side districts afflicted by biting *Cx.p.molestus* despite the absence of larval breeding sites in domestic cisterns and open air tanks in the area. Similarly, a two year survey failed to find *Cx.p.molestus* larvae in tanks containing thousands of gallons of water erected above ground for fire-fighting purposes in the London Underground, despite the presence of other mosquito larvae and the *Cx.p.molestus* biting nuisance below.

Another question is whether or not the STW is the primary or only overwintering site. This was reiterated in the report of another survey conducted in 2006, which examined the incidence of mosquito problems associated with STWs across the country and found that Beckton and Mogden are exceptions rather than the rule.

The implication being that the mosquitoes are not associated with sewage, but rather with specific features of the two sites themselves, likely to be found in other industrial premises. Credence to this viewpoint was provided by one of the earlier reports, when 48 industrial sites had been visited within a 2 km radius of the Mogden STW, which revealed two premises infested with *Cx.p.molestus* during winter (Ismay & Schulten, 2004a).

Detailed studies of the *Cx.p.molestus* biting nuisance in East London were undertaken from November 2003 to August 2006 as a PhD project supported by Thames Water, Barking and Dagenham Borough Council and Newham Borough Council (Curtotti 2007).

The previous year collections of mosquito larvae were made in a residential area of Barking afflicted by mosquito biting nuisance. The larvae were reared to adults and checked for autogeny, which confirmed the presence of *Cx.p.molestus* alongside *Cx.p.pipiens* in three overground sites.

Another human biting mosquito, *An. plumbeus*, was found in one site, but this was to prove quite exceptional. In fact the discovery of overground *Cx.p.molestus* larval breeding sites also proved to be quite rare.

One of the breeding sites was in a channel that normally flows into the river, but had been temporarily blocked by roadworks. The other sites were small and obviously secondary to the channel. The channel was clearly contributing to the biting outbreak in the summer of 2003, but could not have provided an overwintering site.

This is in contrast to two previous biting outbreaks in the area: one in a home for the elderly in Dagenham during the winter of 1978/79 (White, 1980) and the

other on a new housing estate in Beckton between 1992 and 1994 (Cranston *et al*., 1987). In both cases flooding in the basements provided larval breeding sites that survived throughout the winter season until action was taken to remedy the situation.

Many stories and informal enquiries among local residents suggested that mosquito biting in the area had a longer history and was more extensive than indicated by the focal point of complaints in Barking. Residents in Beckton said they were frequently being bitten, but didn't complain because it was normal. This led to the investigation of Beckton STW, where it transpired that mosquito breeding in many areas of the premises was well known among staff and contractors and on occasion pest control firms had been called in.

It appears however that senior management was unaware of the problem and so no concerted action had been taken to deal with it.

Over the period of the study 26 *Cx.p.molestus* larval breeding sites were identified within the STW in the areas that were accessible to the investigator. Parts of the works can only be reached by climbing down ladders above a long drop, or along tunnels filled with noxious gasses. Also certain buildings that are no longer in use, some dating back to the late 19th century, are too dangerous to enter, but certainly contain breeding sites.

Larval density appeared to reach a maximum in the autumn presumably as a consequence of bloodfeeding on human hosts during the late summer. Larvae were found in the area of the returned sludge pumping station in every month throughout the study, whereas other sites, which were widely distributed, were more dynamic.

Sites would dry up and reappear, but also known sites were not always occupied and other seemingly suitable sites were never occupied, despite being adjacent to one that was. Risk evaluations indicated that choice of breeding sites was influenced by water quality, depth and temperature, but larvae could still be found in certain extremes such as clear, cold water in total darkness.

It seems likely that eggs are preferentially laid in already occupied sites. Overall, the underlying cause of the infestation in the Beckton STW is water leakage from cracks in walls and pipes, or poor maintenance or design of equipment. In other words problems that were not entirely specific to an STW and similar to those found in the London Underground and presumably any industrial premises with aging subterranean structures.

Detailed studies were made to examine the dispersal of *Cx.p.molestus* from the STW during the summer. The complaints of biting nuisance were, and remain, much higher north east and east of the Beckton STW, which could simply reflect a more active and vociferous group of complainants, but it is the area where a large overground breeding site was found in 2002/3.

Therefore, surveys were made surrounding, and within, the STW for *Cx.p.molestus* larvae in both natural and artificial breeding sites. Egg rafts were collected and reared in the laboratory and subsequently identified from behavioural and physiological characteristics and by a DNA based diagnostic test. The overall result was almost negative for *Cx.p.molestus*, even when artificial breeding sites were placed in overground locations close to known underground breeding sites. In contrast adults found in houses were all *Cx.p.molestus*.

Similar observations were made during a study of host biting preference, which was carried out on a small (0.4 ha) urban farm located 1.5 km to the east of Beckton STW. Adults of both biotypes were present in large numbers during the late summer, but only the larvae of *Cx.p.pipiens* were found on site.

A survey of 14 industrial premises for underground or very sheltered *Cx.p.molestus* larval breeding sites was also negative. Twelve of these premises were within a triangle formed by placing the points on the farm, the residential area with most biting complaints and the STW. Large drainage systems in the area were also surveyed and despite large collections of *Cx.p.pipiens* only one *Cx.p.molestus* larva was recovered and this was respectively 1.5 and 3 km east of the farm and the STW.

An analysis of the prevailing wind patterns, taking into account direction, speed and height and what is generally known about mosquito host seeking behaviour, flight patterns and passive dispersal by wind, failed to attribute a significant role for the wind in producing the biased distribution of biting complaints.

Overall, despite extensive searches the focal point for the biting problem comes back to the STW.

The theory that most of the biting females are travelling up 1.5 km to obtain a blood meal and then returning to the STW remains intact, but needs to be tested directly. *Cx.p.molestus* certainly has a strong preference for underground, or sheltered, sites, but it is established that on rare occasions this biotype will breed above ground, which may have more to do with chemical rather than physical cues, but needs investigation.

It is also established that other industrial and even residential premises can provide underground larval breeding sites suitable for surviving winter, but none appear to be in the area of the Beckton STW at present.

In this respect it is notable that although records of *Cx.p.molestus* are patchy, it is, or has been, widely distributed in London and across Britain, albeit in highly localised populations. Passive transportation is obviously a key factor in the spread of *Cx.p.molestus* and presumably this occurs with relative ease in London.

To determine host biting preference, bloodfed females were collected on alternative nights over several weeks in the late summer of 2005 and again in 2006 within the confines of the aforementioned urban farm. The samples were taken from the farmer's house, a caravan and a hut housing pigeons and tested for human, mouse, dog and pigeon blood. No *Cx.pipiens* s.l. adults were ever found in the stable or near the horses so this structure was not included in the study.

Cx.p.pipiens was only found in the pigeon hut and only fed on pigeons – the adult females will enter houses occasionally and more so as winter approaches, but appears to never bite humans. Most of the *Cx.p.molestus* were found in the farmer's house or the caravan and about a third in the pigeon hut, but most of the latter were not bloodfed. Overall about eight percent of *Cx.p.molestus* blood meals analysed were from birds and the rest were from humans or dogs. The only other mammal biting mosquito found was *Cs. annulata*, but in small numbers and mostly in the stable.

The results confirm that *Cx.p.molestus* could act as a bridge vector for transmission of arboviruses, such as West Nile, from birds to mammals, but presumably not as efficiently as hybrids found in southern Europe, or the hybrid-like form found in the USA.

In this context it was important to ask if the two biotypes can interbreed in the wild. The data from the study in Scotland and from the London Underground suggested not. Samples of both biotypes from Scotland and from Barking were included in an analysis based on microsatellite DNA, which confirmed that they were very distinct and also showed that they were more closely related to their counterparts abroad than each other.

As would be expected, the *Cx.p.molestus* population from Scotland was highly inbred, but the Barking population was also much inbred relative to *Cx.p.pipiens*. This analysis led to the development of a DNA based diagnostic test, which was evaluated on material from East London and across the country. Nearly 1,500 mosquitoes were tested and produced results consistent with identification based on autogeny and steganomy and no evidence of hybrids was found.

The conclusion made in the most recent study on London Underground *Cx. p.molestus* that this population had evolved from *Cx.p.pipiens* was entirely wrong. The two biotypes arrived in Britain separately and probably several times; as the example in Scotland demonstrates it can happen with relative ease.

What has changed is the totality of the reproductive barrier between the two. In Britain there will only be a few months when the two biotypes physically overlap, at least in an active state. How often, or over what period of time, virgin females would encounter males of the alternative biotype needs to be investigated, but it is clearly going to be limited, especially as shared larval breeding sites appears to be a rare occurrence.

Presumably differences in mating behaviour have become more marked reducing further the scope for interbreeding. Another factor is a difference in *Wolbachia* infection. As already mentioned British *Cx.p.pipiens* carries a *Wolbachia* strain that is not found elsewhere in Europe, whereas the Scottish *Cx. p.molestus* was found with a strain common in other European populations.

An extension of this study to samples from all over Britain including areas of London confirmed that the two biotypes carry different *Wolbachia* strains. This adds further confirmation of the separation of the two biotypes and provides yet another possible mechanism promoting that separation.

In summary, the results provide good news that potential bridge vectors for arboviruses will not include *Cx.p.pipiens*, or hybrids with *Cx.p.molestus*.

The latter must be considered a candidate bridge vector, but not as efficient as it might be and spatially and temporally limited. The inbred nature of the population in the Beckton STW and the rare occurrence of overground breeding sites indicate that *Cx.p.molestus* is relatively tied to its overwintering sites.

On the downside the difficulty in distinguishing the two biotypes and finding *Cx.p.molestus* larval breeding sites presents a challenging control problem.

7.0 Mosquito control

1. Control of British mosquitoes

The Chartered Institute of Environmental Health has produced a comprehensive set of guidance notes entitled *Guidance notes on the management of UK mosquito vectors of disease* (CIEH, 2006).

Many other sources can be found for advice in different contexts such as self defence for individuals or households, or strategies against a specific disease. The guidelines produced by Centers for Disease Control and Prevention (CDC) for West Nile virus control in the USA shortly after the outbreak are an example that reflect recent experience and many recommendations and established practice in other temperate regions for mosquito control (MMWR, 2000).

There is little to be gained from attempting to summarise either here and only a few points are noted. Baseline surveillance data and continued monitoring of mosquito populations are required to establish the need, impact and continued success of mosquito control. This includes accurate identification of species, identification of larval breeding sites and monitoring adults for arboviruses, where it must be noted that trapping has to be effective.Source reduction "remains the most effective and economical method of providing long-term mosquito control in many mosquito habitats". This can be achieved via raising public awareness and public management programmes. Larviciding chemicals labeled for mosquito larviciding in the USA include temephos (Abate), *Bacillus thuringiensis* (Bti), *Bacillus sphaericus*, methoprene and diflubenzuron (Dimilin). Several oils, expanded polystyrene beads and predacious fish (*Gambusia*) are also noted. In France temephos and Bti are used for larviciding. This can offer the greatest impact on the mosquito population, but the consequences of overlooking a single breeding site can be dramatic. Adulticiding employs Ultra Low Volume (ULV) spraying from lorrys or aircraft, thermal fogging, or spraying of residual insecticides on surfaces where mosquitoes are likely to rest. Adulticides labeled for use in the USA include OPs such as malathion, naled and natural pyrethrins and synthetic pyrethroids (permethrin, resmethrin and sumithrin).

For ULV and fogging timing is essential, since they will only kill flying mosquitoes during a short period after application. Repeated applications are needed, in the order of applications 3-4 days apart Aerial spraying achieves maximum coverage, but is only cost effective if used across a very wide area and is limited day-time use. Ground based (from lorrys) will be more cost effective. CDC specifically point out that adulticiding is usually the least efficient mosquito control technique.

Clement Ramsdale and Keith Snow (1995) provide a detailed account of past and current mosquito control operations in Britain and a comprehensive review of the behaviour and habitats of the major man biting species. Little has changed since this was published and descriptions of specific control methods and recommendations for their use are in line with measures being taken in other countries in Europe and the USA. A summary here will not provide a useful substitute for reading this book, but again a few points will be highlighted. In 1985 the authors sent out a short questionnaire to local authorities across the country requesting information on reports of mosquito biting and control efforts. Positive responses were obtained from 81, about half of these included complaints from within the last year and almost all were within the Southern half of England. More than half had undertaken some control in the past and 22 were still engaged in some sort of control at the time.

Fairly detailed accounts of many of the control programmes implemented in the past and including those still ongoing are given. It is notable that almost exclusively the measures taken were source reduction and larviciding.

In more recent years the only larvicide used has been Bti. Many British mosquitoes pass through only a single generation in a year and thus larviciding over a short period only once, or twice, per year is a very cost effective strategy. One example is described where, because of environmental concerns, implementation of larviciding was to be restricted to occasions when larval numbers exceeded an agreed threshold. This arrangement is no longer in practice, but the idea may merit some consideration for locations deemed to be at risk.

A number of biting outbreaks were the direct result of human activity.
For example the building of a new golf course, subsequently required a sustained control programme, whereas problems on a new housing estate were permanently resolved and was therefore a one-off.
A number of species will enter houses to rest and/or to bite, in some cases at a level that may merit the use of mosquito coils or a thermal vaporizer, but with the possible exception of *Cx.p.molestus* control of British mosquitoes is best achieved out of doors.

Ideally this should involve source reduction and larviciding, but in an emergency situation fogging probably in the evening and night may offer some control, depending on the prevailing weather conditions. Mosquitoes normally active in the day will be disturbed into flight by the fogging if used in the vicinity of their resting sites. There are several manufacturers of cold fogging equipment suitable for using on a vehicle and used to dispense an aqueous formulation of a pyrethroid such as the Aqua Reslin type supplied by Aventis. The main suppliers in the USA are Clarke and Dynafog, whereas in Germany SwingTec produce the Mobilstar unit. They all have a large petrol engine and heavy duty blower so are quite expensive. For going into narrow alley ways, there is a knapsack version of the Swingtec equipment while in USA there are some small hand carried units (G. Matthews pers. com). Spraying a residual insecticide in the vicinity of bird roosts in urban areas may have a greater impact on bird biting mosquitoes like *Cx.p.pipiens*.

The evolution of resistance to insecticides is common in mosquitoes. It can reach levels that have serious consequences for control, but its occurrence is not inevitable, its onset and build-up can be delayed and in some instances the consequences may be moderate or negligible. No fully comprehensive search for insecticide resistance has been made on British mosquitoes and with little ongoing mosquito control it might appear unlikely. However, two examples have been illustrated above.

2. Alternative control strategies

Conventional control strategies based primarily on source reduction and the use of insecticides suffer from many disadvantages, not least of which is the environmental impact, the expense and the difficulty of achieving and maintaining adequate coverage, especially when targeting widespread low density populations or populations in inaccessible places.

A number of now well established alternatives can be used to substitute part or all of a mosquito control programme. They are alternative in the sense that they may replace insecticide, but they are fully compatible with integration into a control strategy employing a range of methods. The following are prominent examples.

- Oviposition traps provide an artificial larval breeding site to attract females to lay their eggs. After the eggs hatch the water is drained leaving the larvae stranded and dying. An automated device has been developed that has proved effective against *Aedes* species

- Expanded polystyrene balls are used to form a layer over larval breeding sites preventing adults emerging from pupae or females laying their eggs.
  This is a longer lasting and more flexible alternative to the use of oil and is currently being tried out at Beckton STW

- The sterile insect technique employs the mass release of sterile males to inundate a wild population as much as ten to one. Females only mate once in their lifetime, so if it is with a sterile male, she will not produce viable offspring. This has been a very successful strategy for many agricultural pests and is currently being used against *Ae. albopictus* in Italy and planned for use on the island of La Reunion. It would be ideal for underground populations of *Cx.p.molestus*

8.0 References

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