Project MOSI: rationale and pilot-study results of an initiative to help protect zoo animals from mosquito-transmitted pathogens and contribute data on mosquito spatio-temporal distribution change

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ABSTRACT

Mosquito-borne pathogens pose major threats to both wildlife and human health and, largely as a result of unintentional human-aided dispersal of their vector species, their cumulative threat is on the rise. Anthropogenic climate change is expected to be an increasingly significant driver of mosquito dispersal and associated disease spread. The potential health implications of changes in the spatio–temporal distribution of mosquitoes highlight the importance of ongoing surveillance and, where necessary, vector control and other health-management measures. The World Association of Zoos and Aquariums initiative, Project MOSI, was established to help protect vulnerable wildlife species in zoological facilities from mosquito-transmitted pathogens by establishing a zoo-based network of fixed mosquito monitoring sites to assist wildlife health management and contribute data on mosquito spatio–temporal distribution changes. A pilot study for Project MOSI is described here, including project rationale and results that confirm the feasibility of conducting basic standardized year-round mosquito trapping and monitoring in a zoo environment.
Key-words: attractants; climate change; monitoring; mosquitoes; *Project MOSI*; surveillance; spatio–temporal distribution; wildlife health; zoological networks.

MOSQUITO-RELATED HEALTH ISSUES FOR ZOOS AND SIMILAR FACILITIES

Mosquitoes are the principal vectors of a wide range of diseases, including human and avian malaria, dengue, West Nile encephalitis and filariasis (Becker *et al.,* 2010; Kilpatrick & Randolph, 2012; World Health Organization, 2013a). A range of species has been recorded as succumbing to mosquito-transmitted pathogens in zoos and theme parks. Documented cases include African black-footed penguins *Spheniscus demersus* with avian malaria (Grim *et al*., 2004) and eastern equine encephalitis virus (Tuttle *et al.*, 2005), Great gray owls *Strix nebulosa* with Usutu virus (Weissenböck *et al.*, 2002), Humbolt penguins *Spheiniscus humboldti* with heartworms *Dirofilaria immitis* (Sano *et al.*, 2005), and a Polar bear *Ursus maritimus* (Dutton *et al.*, 2009) and two Orcas *Orcinus orca* with West Nile virus infection (Jett & Ventre, 2013).

Blood-feeding mosquitoes have the ability to track airborne chemicals produced by the vertebrate host to locate them in order to have a blood meal, which is essential for viable egg production in most species (Dekker & Cardé, 2011). The combination of odours varies amongst species and mosquitoes can be more or less attracted to them depending on their feeding preference, even if, at close range, proximity to the host is likely to be more important than species identity (Takken & Verhulst, 2013). Several mosquito species are true 'generalists' as far as host species preference is concerned.

Understanding distribution, population abundance, activity periods and other behaviours of mosquito species helps optimize protection efforts for human, domestic-animal and wildlife populations (Becker *et al.,* 2010; World Health Organization, 2013a). Monitoring and surveillance are key to obtaining such information, and enabling appropriate vector and disease control measures to be taken (Adler *et al.,* 2011; Tuten 2011a; Tuten *et al.,* 2012; World Health Organization, 2012, 2013a), especially when increasing changes in environmental conditions (Barnosky *et al.*, 2012; Hansen *et al.,* 2013) are considered.

MOSQUITO SPATIO–TEMPORAL CHANGES AND ASSOCIATED HEALTH ISSUES

Human activities have long influenced the distribution of many mosquito species (Becker *et al.*, 2010). Historically, this has largely been the result ofhuman induced landscape changes, and inadvertent transportation through the movement of goods and people (Kilpatrick & Randolph, 2012). Some of these changes are positive others negative to mosquito population dynamics. More recently , some dendrophilic species (i.e. mosquitoes that lay their eggs in water-filled tree-holes), such as the Asian tiger mosquito *Aedes albopictus* and *Anopheles plumbeus,* are adapting or changing their behaviour to the human-built landscape by laying eggs not only in water-filled tree cavities but also in artificial small water containers and sewage systems in urban environments (Benedict *et al.*, 2007; Schaffner *et al.*, 2012). The unintentional assistance provided by human activities combined with the great adaptability of many mosquito species has enabled extensive colonization outside of their natural range areas. *A. albopictus* exemplifies how extensive such range expansions can be (Benedict *et al*., 2007; Roiz *et a*l., 2011; Caminade *et al*., 2012).

Anthropogenic climate change (IPCC, 2013) presents a wide range of direct and indirect health impact issues (World Health Organization, 2003, 2009; Patz *et al.*, 2005; Confalonieri *et al.,* 2007; Costello *et al.,* 2009, 2011). The many indirect health issues include vector-borne disease impacts (Sutherst, 2004; Epstein & Mills, 2005; Kurane, 2010; Moore *et al.*, 2012). Paull & Johnson (2013) summarize the complex physiological, range-shift, biotic-interaction and evolutionary challenges of predicting and attributing climate-driven changes to disease dynamics. However, a substantial body of publications and health-agency reports highlights the significance of climate change on vector-borne diseases (Kurane, 2010; Eastwood *et al.*, 2011; Guis *et al.*, 2012; Gallana *et al*., 2013; World Health Organization, 2013b), including actual and projected spatio–temporal changes to mosquito distribution and associated disease issues (Patz *et al.,* 2005; Confalonieri *et al.,* 2007; Paaijmans *et al.,* 2010; Garamszegi, 2011; Roiz *et al.,* 2011; Hongoh *et al.,* 2012; Loiseau *et al.*, 2012; Altizer *et al.*, 2013; Fischer *et al.,* 2013; Gallana *et al.,* 2013; Hueffer *et al.,* 2013; World Health Organization, 2013c).

SURVELLIANCE POTENTIAL OF ZOO AND WILDLIFE-PARK NETWORKS

Barbosa (2009) highlights the role that zoos and aquariums can play in researching the effects of climate change on animal health. Tuten (2011b) highlights the potential early warning role that zoos can provide for the management of mosquito-borne diseases in an era of global climate change. In the context of mosquito research, many zoos have the potential to provide valuable mosquito-monitoring and research opportunities This is largely due to the combination of novel species assemblages that zoos and similar facilities maintain, and the diverse range of microhabitats and shelters suitable for mosquito breeding and overwintering (Adler *et al.*, 2011; Nelder, 2007; Tuten, 2011a; Tuten 2011b; Tuten *et al.,* 2012). Such environments can attract and maintain a wide range of mosquitoes, allowing them to be detected and studied. Zoos and similar facilities often maintain a variety of species outside their natural range areas. Such circumstances can expose naïve or susceptible species to new pathogens, including pathogens native to the local area of the zoo (Adler *et al*., 2011; Tuten, 2011a; Tuten 2011b; Tuten *et al*., 2012). Most zoo animals are routinely monitored for signs of illness and new acquisitions are quarantined. However, as mosquitoes may not be excluded from quarantine animals it is feasible that diseases could be acquired by mosquitoes that have blood fed on already infected animals and carried to other hosts (Tuten 2011b). These considerations make zoos valuable health-surveillance sites (Nelder, 2007; Adler *et al.*, 2011; Tuten 2011b; Tuten *et al.*, 2012) for monitoring native mosquito activity and for detecting non-native mosquito introductions (Ejiri *et al.*, 2011; Tuten, 2011a). The diverse range of species found in most zoos and their relatively close proximity to each other also make zoos valuable places to study the biting behaviour and feeding preferences of mosquitoes (Ejiri *et al.*, 2011; Tuten 2011b; Tuten *et al.*, 2012). Indeed, there already is a considerable record of zoo-focused mosquito study (e.g. Beier & Trpis,1981; Nolen, 2001; Derraik, 2004a,b; McGowan, 2004; Sano *et al.*, 2005; Nelder, 2007; Adler *et al.*, 2011; Ejiri *et al.*, 2011; Tuten, 2011a; Tuten 2011b; Tuten *et al.*, 2012). However, the potential of national, regional and global-level zoo networks to contribute to mosquito-monitoring efforts remains largely unutilized.

There is potential for zoos to improve their animal health management and help identify spatio-temporal distribution changes in mosquito species. To do this, zoos need to conduct basic mosquito monitoring in a standardized and collaborative manner, preferably in liaison with relevant public-health specialists, agencies and initiatives. The World Health Organization's (WHO) *Global Strategy for Dengue Prevention and Control 2012–2020* (World Health Organization, 2012), the WHO European surveillance and control of invasive mosquito vectors and re-emerging vector-borne diseases initiative (World Health Organization, 2013a), and the European Network for Arthropod Vector Surveillance for Human Public Health (VBORNET) (Schaffner, 2012) are examples of current initiatives that zoo-based mosquito monitoring data could potentially be contributing to.

PROJECT MOSI

Responding to the health issues and surveillance potential described above, in October 2010, the World Association of Zoos and Aquariums (WAZA) and the Institute for Zoo and Wildlife Research (IZW), Berlin, Germany, in concert with the Zoological Society of London (ZSL) and Imperial

College, UK, agreed to develop a permanent zoo-based mosquito-monitoring programme: *Project MOSI* (Mosquito Onset Surveillance Initiative). Focusing on the monitoring potential of the world's zoo and wildlife-park networks, the core remit of this initiative is to help protect vulnerable wildlife species from mosquito-transmitted pathogens, through improved knowledge of mosquito-species composition, population abundance and seasonal activity at the location of the monitoring traps. This information could, for example, help optimize prophylactic veterinary treatments and mosquito-control efforts (Silver, 2008; Becker *et al*., 2010; Tuten, 2011a; Kroeger *et al.*, 2013) and also contribute data to relevant mosquito and public-health specialists, agencies and surveillance initiatives.

PILOT-STUDY METHODS AND RESULTS

The *Project MOSI* initiative was informed by a range of monitoring activities on the ZSL London Zoo site from 2005 onwards. In 2005, a single Mosquito Magnet trap was placed in the flamingo enclosure which, at that time, was also temporarily holding African black-footed penguins. The trap was set up in response to cases of avian malaria in the penguins, with the aim of investigating which mosquito species were present in the enclosure and possibly be involved in the transmission of this disease. The Mosquito Magnet was fitted with the standard mosquito attractant combination of CO_2 (mimicking breath) and Octenol (a chemical preparation designed to mimic mammal sweat). Adult mosquitoes of *Culex pipiens*, *Culiseta annulata* and *An. plumbeus* were collected (see Box 1).

In conjunction with the Mosquito Magnet trapping, a survey of potential mosquito larval sites in the grounds of ZSL London Zoo, and testing of different trapping methods (e.g. resting boxes and gravid traps), was conducted during the summer of 2005. The main water bodies within the zoo site that were capable of harbouring mosquito larvae were mapped and monitored weekly from July to September 2005. Thirteen water bodies were found to contain mosquito larvae, with *Culex pipiens* being the predominant species and a small number of *Culi. annulata* also being found (Fig. 1). No *Anopheles plumbeus* larvae were found in the ZSL London Zoo grounds, despite searching tree cavities filled with water, which constitute the main larval environment for this species. It was therefore suspected that trapped *A. plumbeus* adults originated from the surrounding Regent's Park area of public parkland which provides better larval sites for this species.

Twelve resting boxes were built in spring 2005 (following Crans, 1989) and tested over the summer. Only two boxes were regularly found with resting females inside and it was later discovered that these two boxes had been inadvertently located near natural resting places. These resting places were subsequently regularly monitored for gravid and blood-fed females, and the use of resting boxes was abandoned due to the lack of positive results. Tuten (2011b) reported similar results with resting boxes in a separate zoo survey initiative. In 2005 two gravid traps (Allan

& Kline, 2004) were deployed with a hay infusion as the attractant. This was effective in attracting gravid *Culex pipiens* but failed to attract gravid *Culiseta annulata* or *Anopheles plumbeus* (Fig. 2) even when the attractant infusion was modified in an attempt to match the needs of these species (water with bird faeces for *Culiseta annulata* and a leaf infusion for *Anopheles plumbeus*). Because resting gravid *Culex pipiens* can easily be collected in resting places on the Zoo premises and are regularly found in Biogents Mosquitaire traps (see below) the use of gravid traps was discontinued.

Year-round mosquito monitoring at the ZSL London Zoo site commenced in 2008 with three Mosquito Magnet traps fitted with the CO_2 and Octenol attractants. One trap was located near a newly constructed penguin enclosure (Fig. 3). A Mosquito Magnet trap was also installed in the flamingo enclosure (Fig. 4) and in a mixed-bird species exhibit called the Snowdon Aviary (Fig. 5). Trapped mosquitoes were collected once a week and morphologically identified to species level using appropriate keys (Snow, 1990). Results to date indicate that the local mosquito population consists mainly of *Culex pipiens, Culiseta annulata* and *Anopheles plumbeus* (Fig. 6).

In 2010, Biogent Mosquitaire traps (Meeraus *et al.*, 2008; Schmaedick *et al.*, 2008; Becker *et al.*, 2010) were utilized in a standardized manner. These traps use a lactic-acid attractant (designed to mimic human sweat) and were developed specifically to attract the tiger mosquito *Aedes albopictus*. At ZSL London Zoo these traps attracted larger numbers of *Culex pipiens* relative to the CO_2 and Octenol baited Mosquito Magnet traps. *Culiseta annulata* was also found in the Mosquitaire traps as were a small number of *Anopheles plumbeus* (Figs 3, 4 and 5).

In addition to capturing large numbers of *C. pipiens*, relative to the Magnet traps, in which none of the mosquitoes were gravid or blood fed, 80–90% of the Biogents Mosquitaire-trapped *C. pipiens* appeared to be gravid and some were also blood-fed.   At least in the case of *C. pipiens* (the most common species found on the ZSL London Zoo site) the Biogents Mosquitaire traps proved the more effective monitoring option. These traps also are cheap and easy to run, and appear to fill the role of a gravid trap, at least for *C. pipiens*. Our results from ZSL London Zoo led to the Biogents Mosquitaire traps being adopted as the standard *Project MOSI* monitoring trap from 2010 onwards.

In spring 2011, a new penguin enclosure was built on the site of the earlier penguin exhibit. This new exhibit held a much larger number of birds (up to 90 animals) and a wider range of species, including Humboldt penguin *Spheniscus humboldti*, African black foot penguin and a single Northern rockhopper penguin *Eudyptes chrysocome moseleyi*. According to Cummins *et al.* (2012) the biting rate of mosquitoes per host is higher for dispersed groups of hosts compared with more compact groups. Relative to the old enclosure, the new Penguin Beach exhibit displays more animals scattered over a larger area and perhaps this could attract more mosquitoes. In response, additional Biogents Mosquitaire and Mosquito Magnet traps were installed between the penguin enclosure and the fence line of the Zoo (Plate 1). Following historic avian malaria cases in the penguins at the Zoo and fresh cases occurring in summer 2012, together with *C. pipiens* still being trapped in mid-November, an investigation of indoor and outdoor overwintering adults was carried out in January–February 2013.

Overwintering *C. pipiens* females collected in and near animal enclosures at ZSL London Zoo are often found to be full of eggs and in January 2013 two resting females (in a bird enclosure) had had visible blood meals, indicating that they were still active at this time of the year. The 2012 season trap catches confirmed *C. pipiens* winter activity in November 2012 and January 2013 but no *C. pipiens* were found in traps during December 2012 or over the previous winters (2008–2011). The summer 2012 penguin-enclosure traps captured ten times as many *C.pipiens* relative to previous trapping summers while *Culiseta annulata* were captured in similar numbers as previously and *Anopheles plumbeus* in lower numbers than previous summers (Fig. 6). It remains to be determined (pending molecular analysis) whether these *C. pipiens* are of the *pipiens or molestus* morph, or a mixture of these (Fonseca *et al.*, 2004).

STATISTICAL ANALYSES

For the 2005 breeding-site data numbers at each site were compared to what would be expected by chance (i.e. equivalent numbers at every site) using a likelihood test for goodness of fit (re). Tests for trends in numbers of individuals over years were made using both linear and quadratic regression. Because the numbers of traps varied between years the total number of captures was divided by the number of traps for each year. A chi-square contingency test was used for the comparison of attractants, treating individual mosquitoes as replicates. As the attractants are what was manipulated in this experiment it can be argued that the trap itself is the experimental unit and that several traps with each attractant would be required. This was not possible in the current study so the individual-based analysis is presented with the caveat that trap-level replication is required in the future for more robust assessment. All analyses were carried out using JMP Version 10 (JMP®, Version 10. SAS Institute Inc., Cary, NC, 1989-2007).

FULL PROGRAMME IMPLEMENTATION AND MONITORING PROTOCOL

Following the encouraging pilot-study results at ZSL London Zoo, the *Project MOSI* initiative has now been rolled out to Copenhagen Zoo, Denmark, and Artis Royal Zoo in Amsterdam, the Netherlands, and a number of additional institutions are also in the process of initiating the implementation of this project. The *Project MOSI* protocol has been established in order to ensure standardarsation of methods across participating institutions. This protocol is as follows:

- Trap model: Biogent Mosquitaire.

- Attractant: Biogent Sweetscent (lactic acid).
- Weekly collection of trapped mosquitoes.
- Samples stored in a fridge at 4°C prior to identification.
- Species identification (including collaborations with relevant specialists).
- Blood-fed and gravid-specimen storage (where feasible) in −80°C freezer for potential future analyses of host species and pathogen carriage.

DISCUSSION

The *Project MOSI* pilot study confirmed the feasibility and relative ease of conducting a standardized, year-round mosquito monitoring programme in a zoo environment, provided the necessary mosquito-identification skills are available or can be accessed. The weekly trap specimen collection protocol also proved valuable in helping to optimize timing of prophylactic antimalarial treatments for the vulnerable penguins at ZSL London Zoo. In addition to providing data on the mosquito species complement and weekly activity levels at the ZSL London Zoo trap location, the adult mosquito trapping and resting surveys informed the need for mosquito-control efforts.

The practical considerations associated with realising a zoo community-based monitoring initiative, such as *Project MOSI,* requires trapping and monitoring demands are to be as straightforward, cost effective and easy to follow as possible. In this regard the Biogents Mosquitaire traps have proved very successful as they are relatively inexpensive to purchase and run, and are easy to maintain.

As expected, a longer time frame is necessary for identifying any significant spatio–temporal changes. Provided the year-round trapping protocol is adhered to (i.e. collection data continues to accumulate into the future), analysis of catch data over longer time periods should always be possible. The apparent attraction of *Ades albopictus*, *Culex pipiens* and *Culiseta annulata* to feathers of, some or all, of the ZSL London Zoo penguin species (see Box 2, Fig. 7) suggests that the potential mosquito-attractant properties of penguin, and other bird, feathers merits further study.

At ZSL London Zoo, the identification of specimens proved to be straightforward thanks to the available mosquito-identification skills and reference material (Snow 1990). However, as addressed in Adler (2011) and Tuten 2011b ensuring sufficient entomological skills are available for identifying such specimen material is the greatest practical challenge for many zoos. Institutions with the relevant 'in house' entomological skills, or with the ability to access such skills (e.g. by collaborating with museums, health facilities or mosquito control districts as demonstrated in Tuten 2011b) are obviously best placed to participate in such monitoring initiatives (Tuten, 2011b)The identification challenge can also be addressed by several participating institutions sharing an identification specialist, as is the situation with ZSL London Zoo, Artis Royal Zoo and Copenhagen Zoo. The advantages of developing specimen-identification capacity can extend beyond mosquitoes to a wide range of arthropods important to medicine or veterinary medicine with associated health-management benefits (Adler *et al.*, 2011; Nelder, 2007).

The development priorities for Project MOSI are to increase the number of participating institutions across zoo and wildlife-park networks, and to liaise with a wider range of specialists, agencies and surveillance initiatives. The limitations of the *Project MOSI* monitoring initiative are acknowledged. The relatively basic level of monitoring involved (i.e. a single trap maintained year round) is insufficient for a participating zoo to establish anything approaching comprehensive site-level mosquito profiles. Such a task would necessitate much greater monitoring and research effort (Tuten 2011b). The rationale for the less demanding monitoring remit of the *Project MOSI* initiative is a practical trade-off between what is technically desirable and what is realistically achievable in terms of implementing an ongoing coordinated zoo-based monitoring programme. The monitoring demands on participating institutions need to be sufficiently modest to encourage initial engagement and ongoing commitment. It is hoped that participation will, over time, further encourage zoos to undertake more robust site monitoring and research initiatives.

How best to standardize for geographic area and species density/compliment is an important protocol requirement as is an ongoing review of additional trap types and attractant options for improved site-level monitoring ability. Optimizing trap-location potential for protecting particularly vulnerable species is another priority. The relative value of including temperature and other environmental data associated with mosquito-trap collection data, against the increased burden this would place on participating institutions, needs to be investigated. McNamara (2007) has highlighted the potential of the zoo community's Zoological Information System (ZIMS) for providing valuable bio-surveillance animal-health data. Adding such location -trapping data onto ZIMS could further enhance the database's bio-surveillance potential.

Whilst acknowledging that direct comparisons cannot be made, the UK moth-trapping programme initiative of the Rothamsted Insect Survey (Harrington & Woiwod, 2007) demonstrates how valuable a permanent network of standardized monitoring traps can be for improving knowledge about species abundance, distribution and changes over time (Conrad *et al*., 2004). Another, more recent, example is the UK surveillance network for *Culicoides* midges' which also utilises a network of single trap sites (M. England, Pirbright Institute, personal communication). The health issues associated with mosquitoes make the case for zoos increasing their attention and monitoring effort on these insects all the more compelling.

CONCLUSIONS

The health-related considerations of disease-vector mosquito species, combined with the need to better understand the exacerbating influence of increasing environmental change on these species, are a compelling rationale for zoos and wildlife parks to monitor and, where necessary, manage mosquito related health threats on their sites. The collective potential of these global zoological networks for assisting wildlife health management and conservation planning (Redford *et al.*, 2012) is considerable especially with sufficient collaborations with relevant entomological specialists, surveillance initiatives (e.g. ECDC, 2009, 2010; World Health Organization, 2012, 2013a) such as the European Network for Arthropod Vector Surveillance for Human Public Health (VBORNET: http://vbornet.eu) and the British Mosquito Recording Scheme (http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/Mosquitoes/MosquitoRecordingScheme/).

An important additional consideration for zoos is their tremendous public-engagement ability and associated potential for raising public awareness of the significance of vector-borne diseases and the importance of effective surveillance and control initiatives.

Despite its acknowledged limitations, the *Project MOSI* initiative provides a realistic opportunity for zoos and similar facilities to improve their current engagement with mosquito monitoring and associated health management and research and to start realising the collective potential of the international zoo networks.

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PRODUCTS MENTIONED IN THE TEXT

Biogents Mosquitaire: mosquito traps, manufactured by Biogents AG, Weißenburgstrasse 22, 93055 Regensburg, Germany.
JMP: statistical discovery software, manufactured by SAS, Cary, NC 27513, USA.
Mosquito Magnet®: mosquito traps, manufactured by Woodstream Corp., 69 North Locust Street, Lititz, PA 17543, USA.
Octenol Biting Insect Attractant: attractant for use with Mosquito Magnet, manufactured by Woodstream Corporation, Lititz, PA 17543, USA.
Sweetscent: lactic-acid attractant, manufactured by Biogents AG, Weißenburgstrasse 22, 93055 Regensburg, Germany.

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Figure and table legends:

Objective
1. Utilize the global zoo network to establish permanent mosquito monitoring trap sites.
2. Help clarify local mosquito species composition, abundance and activity profiles at the trap locations.
3. Help monitor changes in species composition, abundance and activity profiles at the trap locations.
4. Where feasible, preserve trapped blood-fed mosquito specimens for potential host-species clarification and disease investigations.
5. Assist evaluation and management of mosquito-transmitted pathogen threats in the zoo environment.
6. Inform development of mosquito attractants.

Table 1. The principle objectives of Project MOSI (Mosquito Onset Surveillance Initiative). The World Association of Zoos and Aquariums, and the Institute for Zoo and Wildlife Research (IZW), Berlin, Germany, in concert with the Zoological Society of London (ZSL) and Imperial College, UK, collaborated to develop a permanent international mosquito-monitoring programme to help protect zoo animals from mosquito-transmitted pathogens and contribute data on mosquito spatio-temporal distribution change.

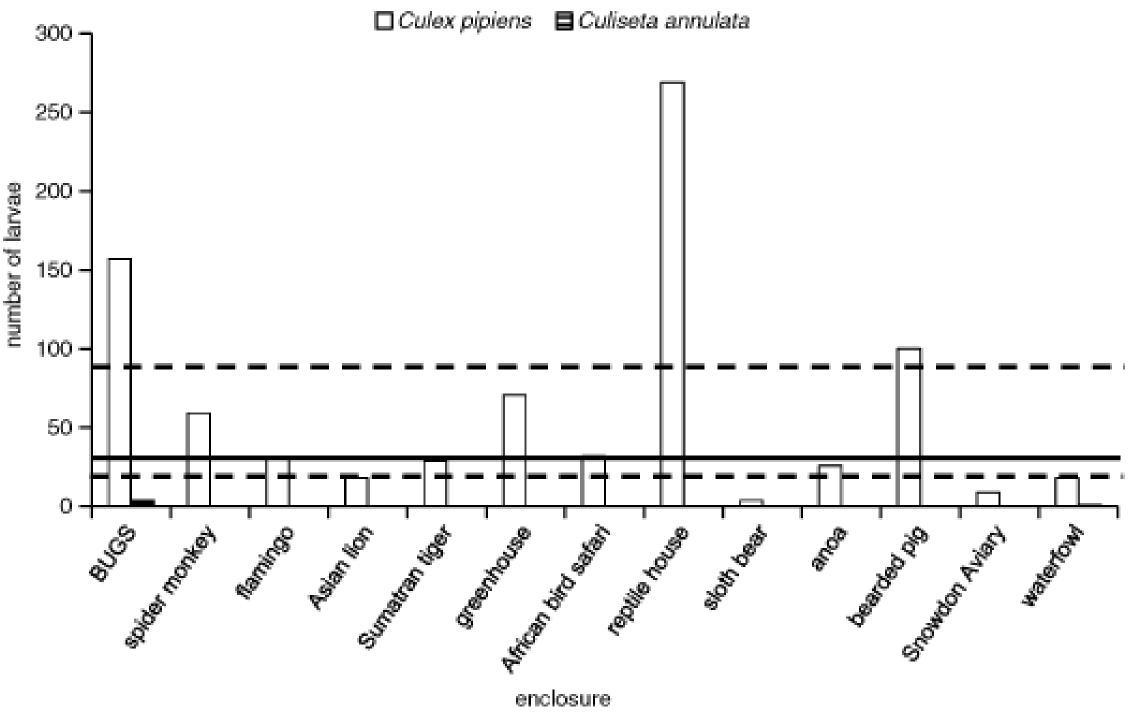

Fig. 1. Number of larvae from each species of mosquito found at each breeding site (see text) during a survey carried out at ZSL London Zoo, UK, in summer 2005. The distribution of *Culex pipiens* **across enclosures deviates from what would be expected by chance (**X^2**=1069·14,** P **< 0·0001). Median is 31, 25%ile is 18, 75%ile is 85. The solid line is at the median and dashed lines at the 25th and 75th%iles on the graph.**

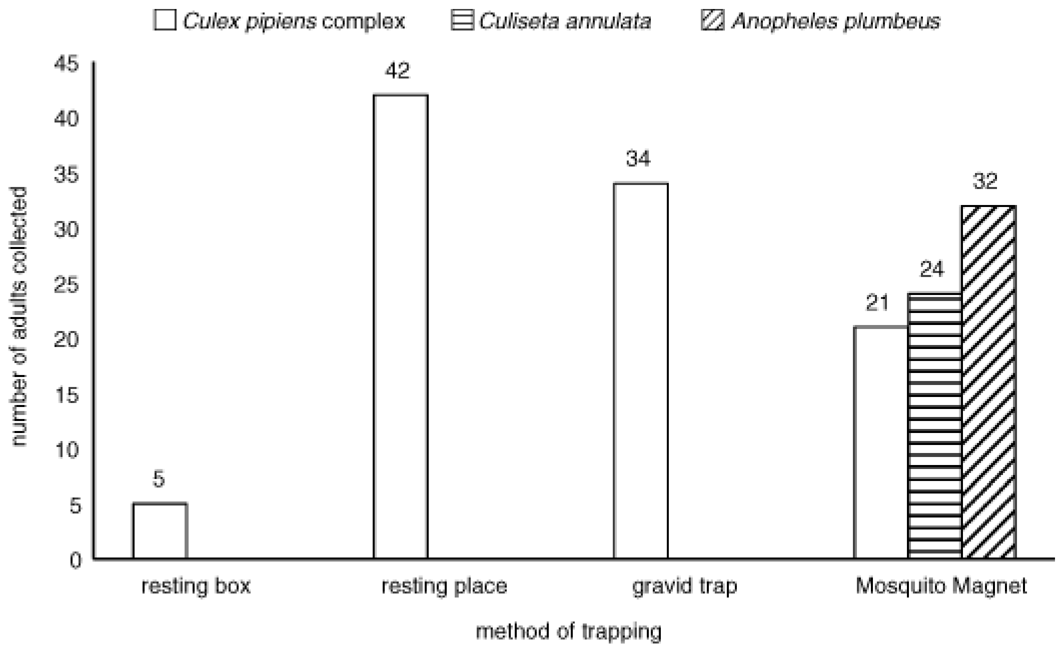

Fig. 2. Three species of adult mosquito were collected during a survey carried out at ZSL London Zoo, UK, in summer 2005 using four different trapping methods.

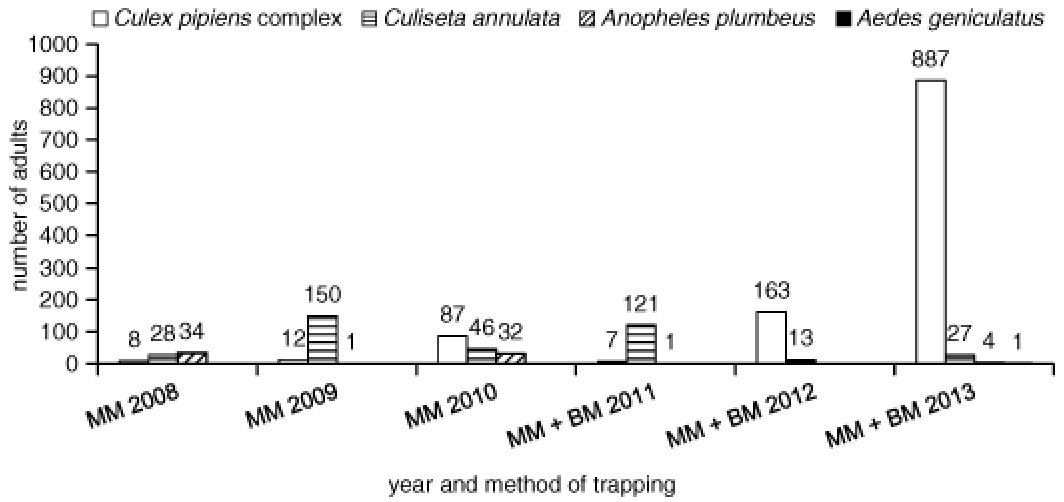

Fig. 3. Total number of adult mosquitoes of each species captured each year (2008–2013) in the penguin enclosure at ZSL London Zoo, UK. Two types of trap were used: MM. Mosquito Magnet; BM. Biogents Mosquitaire. Sometimes the two traps were running concurrently (MM + BM). None of the species show a significant linear or quadratic trend over time. *Culex pipiens* **is closest to showing an increasing trend.**

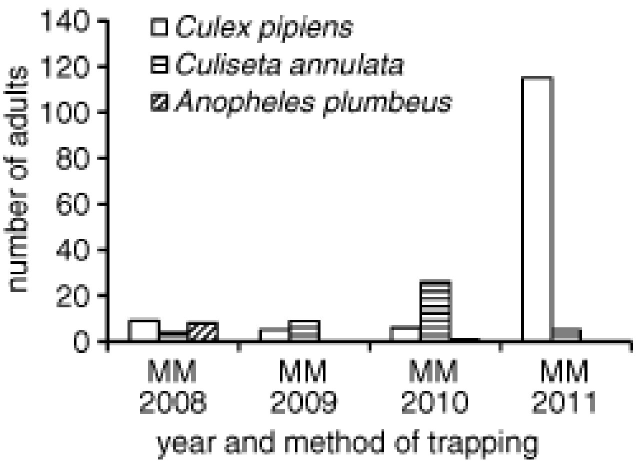

Fig. 4. Total number of adult mosquitoes of each species captured each year (2008–2011) in the flamingo-pond trap location at ZSL London Zoo, UK: MM. Mosquito Magnet trap. None of the species show a significant linear or quadratic trend over time.

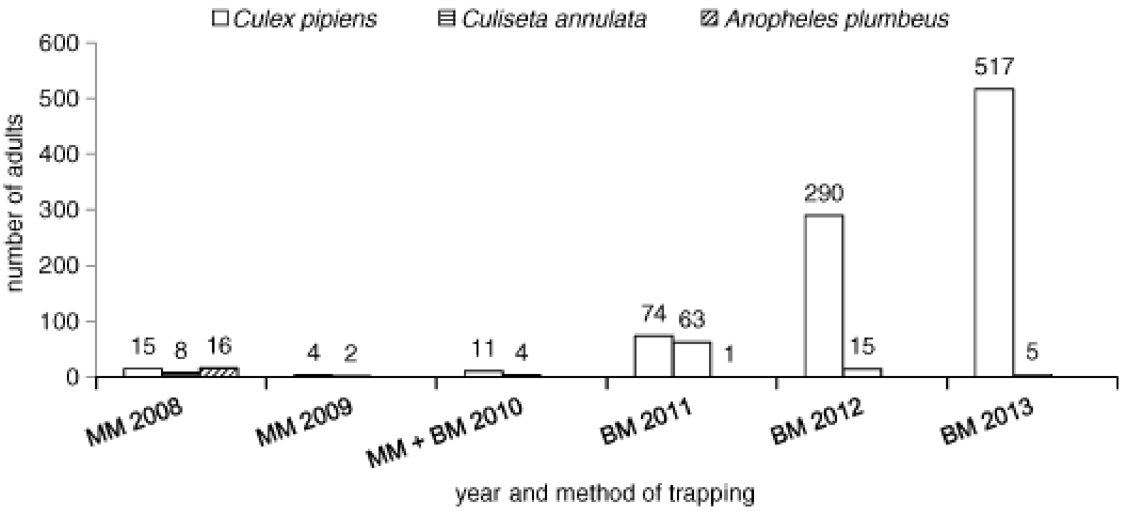

Fig 5. Total number of adult mosquitoes of each species captured each year (2008–2013) in the Snowden Aviary trap location. Two types of trap were used: MM. Mosquito Magnet; BM. Biogents Mosquitaire. Sometimes the two traps were running concurrently (MM + BM). *Culex pipiens* **shows a significant line (R^2 = 0·77, P = 0·022) and quadratic (R^2 = 0·99, P = 0·0008) increasing trend over years. Neither of the other species show any significant trends over time. Note that the significant increasing trend in** *C. pipiens* **only occurs in 2011, 2012 and 2013, and is not influenced by the change in trap type. The data are linear for 2011, 2012 and 2013: R^2 = 0·99, P = 0·0091).**

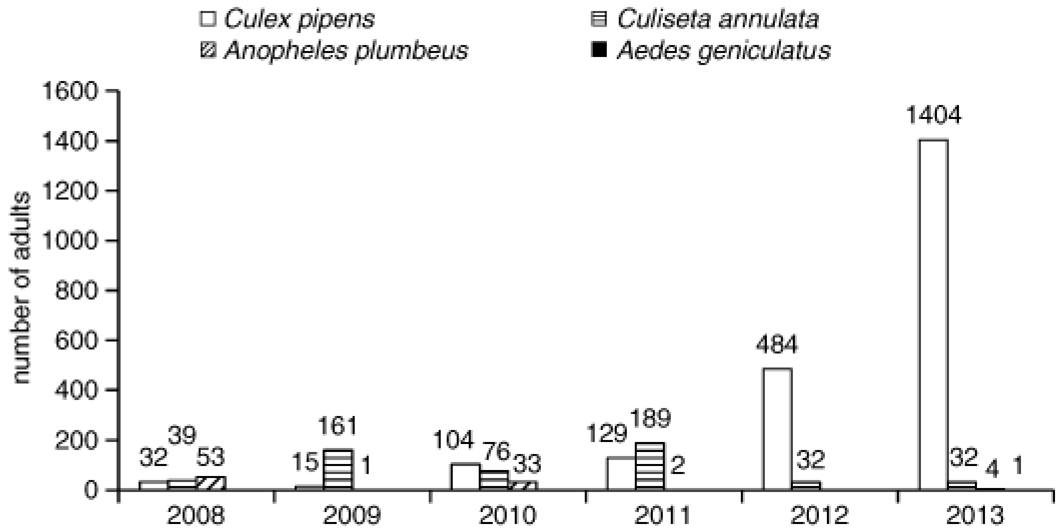

Fig. 6. Number of mosquito adults of each species captured each year (2008–2013) at ZSL London Zoo, UK. *Culex pipiens* **shows a significant linear (*R^2* = 0·66, *P* = 0·049) and quadratic (*R^2* = 0·87, *P* = 0·022) increasing trend over years. None of the other species show a trend over time.**

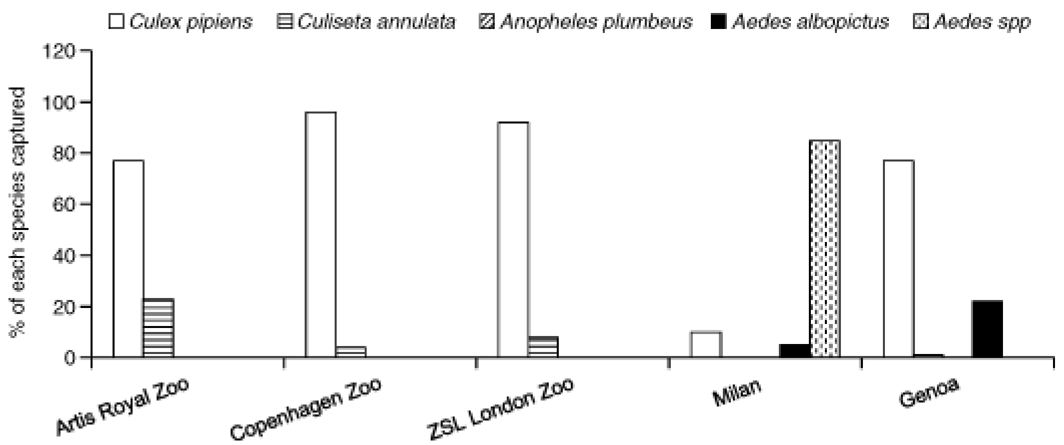

Fig. 7. Percentage breakdown of each mosquito species found at each participating institutions during 2012 for *Project MOSI***. Data from Milan relate to resting adults collected in the grounds of the Department of Veterinary Science and Public Health, University of Milan, Italy. Data from Genoa refers to Biogents Mosquitaire traps set up in a private garden in Genoa, Italy, as described in Box 2.**

Box 1. Mosquito species collected during the *Project MOSI* pilot study .

Asian tiger mosquito *Aedes albopictus*
This forest-living, dendrophilic species (i.e. a mosquito that lays its eggs in water-filled tree-holes) has been inadvertently spread around the world (largely via the used-tyre and tropical-plant trades) and is now established in many cities outside of its natural range, where elevated temperatures, humidity and artificial water pools have enabled it to thrive (Pluskota *et al.*, 2008; Roiz *et al.*, 2011). This species can transmit a number of pathogens of public-health importance, including West Nile virus, yellow fever virus, St Louis Encephalitis virus, dengue fever virus (Fontenille & Toto, 2001) and chikungunya fever virus. An outbreak of chikungunya fever (a disease originally endemic to East Africa) in Italy demonstrates that the introduction of mosquito vectors, such as the Asian tiger mosquito, can eventually be followed by their associated pathogens (Angelini *et al.*, 2007; Bonilauri *et al.*, 2008).

Anopheles plumbeus
Widely distributed throughout Europe, the northern Caucasus, Middle East south to Iran and Iraq, and North Africa, this dendrophilic species has adapted to breed in a range of artificial sites and, as a consequence, has greatly increased in numbers and area over the last few decades with incursion into urban and suburban areas (Dekoninck *et al*., 2011). As a result of its aggressive biting behavior and locally increased abundance, this mosquito has become a significant nuisance and a potential health threat (Schaffner *et al.*, 2012). For example, in Germany two cases of autochthonous (i.e. locally caught) *Plasmodium falciparum* malaria have recently occurred, apparently as a result of transmission by indigenous *Anopheles plumbeus* (Krüger *et al.*, 2001).

Culiseta annulata
Extending into North Africa, Asia Minor and south-west Asia (Becker *et al.*, 2010), this species can thrive in a variety of natural and artificial water conditions, especially nitrogen-rich waters. Females will feed indoors and outdoors on a variety of hosts, including humans and birds (Snow, 1990). Adults overwinter in natural shelters but also human dwellings such as cellars and also domestic-animal buildings where they can be very annoying when their hibernation is interrupted by rising temperatures or humidity (Becker *et al.*, 2010). *Culiseta annulata* can transmit myxomatosis and avian malaria (Gustevich *et al*., 1974), and is also a potential vector of Tahyna virus (Ribeiro *et al*., 1988).

***Culex pipiens* complex**
One of the most widely distributed mosquitoes, *Culex (Culex) pipiens* is part of the *C. pipiens* complex, which is a group of morphologically and evolutionarily closely related mosquitoes with a long association with humans (Vinogradova, 2000). They play important roles in the transmission of several human pathogens including West Nile virus (Epstein & Causey, 2005), St Louis encephalitis virus and lymphatic filarial worms (Reisen *et al.*, 1992; Bogh *et al.*, 1998; Turell *et al.*, 2005; Gomes *et al.,* 2012). They also act as vectors of wildlife pathogens, such as avian malaria *Plasmodium* spp (Woodworth *et al.*, 2005) and West Nile virus (Mereu Piras *et al.*, 2012).

Box 2. Study into penguin feathers versus standard trap attractants.

Observation that most of the mosquitoes trapped at ZSL London Zoo, UK, were collected in traps located by the penguin enclosure raised questions as to what was attracting mosquitoes to that area. Several mosquito species are attracted by bird hosts for which the main attractant seems to be the preen-gland secretion that birds spread on their feathers to render them waterproof (Allan *et al.*, 2006). Harvesting preen-gland extract from penguins would pose ethical issues so moulted feathers were used to investigate whether penguin feathers acted as an attractant for *Culex pipiens* and other mosquito species.

For this trial, two *Biogents Mosquitaire* traps were established in a private garden in Genoa, northwest Italy, in September 2011. This location was chosen because of the absence of live penguins in the area and also to remove the multiple-trap attraction factor that may also account for the greater attraction of the penguin enclosure area relative to the other trap locations at ZSL London Zoo. Genoa was also chosen for this trial because it has a mosquito population that is active over a greater part of the year than in the UK and in greater abundance, thus providing better opportunities for such a comparison trial. Another reason for selecting Genoa was that since 1990 Genoa has been colonized by the Asian tiger mosquito *Aedes albopictus,* making this location an interesting prediction model for expected colonization by nonnative species to other European countries.

One of the two Mosquitaire traps was baited with the standard lactic-acid attractant (Sweetscent), which has been especially developed to attract *Aedes albopictus*, while the other trap was baited with penguin feathers (placed in a net container of comparable size to the lactic-acid attractant and positioned where the normal attractant would usually be located). Surprisingly the two traps attracted a similar number of *Culex pipiens* and *Aedes albopictus* even though *Aedes albopictus* is known to show a preference for mammals over birds and the Sweetscent lactic-acid attractant was specifically designed to attract this species. Even more surprisingly, penguin feathers maintained their attractiveness (if at a decreasing degree) over the following months without being replaced or supplemented with fresh feathers (while the Sweetscent attractant was replaced every 2 months).

Without a third empty trap with no attractant acting as a control, any interpretation of the results is only speculative. However, the potential significance of such novel attractants merits further investigation. Preen-gland compounds found in penguins have been described (Jacob, 1976) and the secretions of each penguin species has a different chemical composition. Further study may prove useful for improving mosquito-control efforts by determining which of the chemical compounds are capable of attracting mosquitoes and how they may vary for different mosquito species.