

Persistent mosquito fogging can be detrimental to non-target invertebrates in an urban tropical forest

Nicole SM Lee ^{Corresp., 1}, Gopalasamy R Clements ², Adeline SY Ting ¹, Zhi H Wong ³, Sze H Yek ^{Corresp. 1}

¹ School of Science, Monash University Malaysia, Bandar Sunway, Selangor, Malaysia

² Department of Biological Sciences and Jeffrey Sachs on Sustainable Development, Sunway University, Bandar Sunway, Selangor, Malaysia

³ Malaysia Immersion Hub, Monash University Malaysia, Bandar Sunway, Selangor, Malaysia

Corresponding Authors: Nicole SM Lee, Sze H Yek

Email address: nlee0010@student.monash.edu, yek.szehuei@monash.edu

Background: Human population growth has led to biodiversity declines in tropical cities. While habitat loss and fragmentation have been the main drivers of urban biodiversity loss, man-made interventions to reduce health risks have also emerged as an unintentional threat. For instance, insecticide fogging to control mosquito populations has become the most common method of preventing the expansion of mosquito-borne diseases such as Dengue. However, the effectiveness of fogging in killing mosquitoes has been called into question. One concern is the unintended effect of insecticide fogging on non-target invertebrates that are crucial for the maintenance of urban ecosystems. Here, we investigate the impacts of fogging on: 1) target invertebrate taxon (Diptera, including mosquitoes); 2) non-target invertebrate taxa; and 3) the foraging behavior of an invertebrate pollinator taxon (Lepidoptera) within an urban tropical forest.

Methods: We carried out fogging with Pyrethroid insecticide (Detral 2.5 EC) at 10 different sites in a forest situated in the state of Selangor, Peninsular Malaysia. Across the sites, we determined the proportion of knocked-down invertebrates and identified them based on morphology to different taxa. At each site, we used a Bayesian statistical framework to determine whether there were credible differences in: 1) the mortality of target invertebrate taxon (Diptera) 3-hr post-fogging; 2) the mortality of selected non-target invertebrate taxa 3-hr post-fogging; and 3) the occurrence of Lepidoptera 1-hr pre-fogging and 1-day post-fogging.

Results: A total of 1874 invertebrates from 19 invertebrate orders were knocked down by fogging treatment across the 10 sites. 3-hr post fogging, 72.7% of the invertebrates were found dead. While we found credible differences in dead vs. alive Dipteran individuals, only 8.8% of the knocked-down invertebrates consisted of Diptera. Among 9 selected non-target invertebrate orders, there were credible differences in dead vs. alive individuals for 3 orders (Araneae, Collembola and Psocoptera), all of which are soft-bodied. In our pre- and post-fogging treatment for Lepidoptera, we also found credible differences in the number of individuals, with less individuals recorded within the vicinity of each site post-fogging.

Discussion: Our results demonstrate that fogging has detrimental effects on non-target invertebrate orders, especially 'soft' bodied and pollinator species. While fogging is effective in killing the target order (Diptera), no mosquitos were collected in the experiment. Hence, the effectiveness of the insecticide to mosquitos remains to be explored. Hard-bodied invertebrates appear to be more 'resistant' to fogging. However, there might be long-term physiological effects. In order to maintain urban biodiversity, we recommend that health authorities and the private sector move away from insecticide fogging and to explore alternative measures to control adult mosquito populations.

Persistent mosquito fogging can be detrimental to non-target invertebrates in an urban tropical forest

Nicole Sze Mei Lee¹, Gopalasamy Reuben Clements², Adeline Su Yien Ting¹, Zhi Hoong Wong³, Sze Huei Yek¹

¹ School of Science, Monash University Malaysia, Bandar Sunway, Selangor, Malaysia

² Department of Biological Sciences and Jeffrey Sachs on Sustainable Development, Sunway University, Bandar Sunway, Selangor, Malaysia

³ Malaysian Immersion Hub, Monash University Malaysia, Bandar Sunway, Selangor, Malaysia

Corresponding Authors:

Sze Huei Yek¹

Email address: yek.szehuei@monash.edu

[Nicole Sze Mei Lee¹](#)

Email address: nlee0010@student.monash.edu

Abstract

Background: Human population growth has led to biodiversity declines in tropical cities. While habitat loss and fragmentation have been the main drivers of urban biodiversity loss, man-made interventions to reduce health risks have also emerged as an unintentional threat. For instance, insecticide fogging to control mosquito populations has become the most common method of preventing the expansion of mosquito-borne diseases such as Dengue. However, the effectiveness of fogging in killing mosquitoes has been called into question. One concern is the unintended effect of insecticide fogging on non-target invertebrates that are crucial for the maintenance of urban ecosystems. Here, we investigate the impacts of fogging on: 1) target invertebrate taxon (Diptera, including mosquitoes); 2) non-target invertebrate taxa; and 3) the foraging behavior of an invertebrate pollinator taxon (Lepidoptera) within an urban tropical forest.

Methods: We carried out fogging with Pyrethroid insecticide (Detral 2.5 EC) at 10 different sites in a forest situated in the state of Selangor, Peninsular Malaysia. Across the sites, we determined the proportion of knocked-down invertebrates and identified them based on morphology to different taxa. At each site, we used a Bayesian statistical framework to determine whether there were credible differences in: 1) the mortality of target invertebrate taxon (Diptera) 3-hr post-fogging; 2) the mortality of selected non-target invertebrate taxa 3-hr post-fogging; and 3) the occurrence of Lepidoptera 1-hr pre-fogging and 1-day post-fogging.

Results: A total of 1874 invertebrates from 19 invertebrate orders were knocked down by fogging treatment across the 10 sites. 3-hr post fogging, 72.7% of the invertebrates were found dead. While we found credible differences in dead vs. alive Dipteran individuals, only 8.8% of the knocked-down invertebrates consisted of Diptera. Among 9 selected non-target invertebrate orders, there were credible differences in dead vs. alive individuals for 3 orders (Araneae, Collembola and Psocoptera), all of which are soft-bodied. In our pre- and post-fogging treatment for Lepidoptera, we also found credible differences in the number of individuals, with less individuals recorded within the vicinity of each site post-fogging.

53 **Discussion:** Our results demonstrate that fogging has detrimental effects on non-target
 54 invertebrate orders, especially ‘soft’ bodied and pollinator species. While fogging is effective in
 55 killing the target order (Diptera), no mosquitos were collected in the experiment. Hence, the
 56 effectiveness of the insecticide to mosquitos remains to be explored. Hard-bodied invertebrates
 57 appear to be more ‘resistant’ to fogging. However, there might be long-term physiological
 58 effects. In order to maintain urban biodiversity, we recommend that health authorities and the
 59 private sector move away from insecticide fogging and to explore alternative measures to control
 60 adult mosquito populations.

Introduction

Urban biodiversity is expected to decline under current human population growth rates. More than half of the world's population now resides in cities (Zhang 2016) – this is likely to lead to massive land development and consequently, greater rates of natural habitat loss and fragmentation (Clark, Reed & Chew, 2007). While urbanization has led to the decline of certain invertebrate taxa (Eisenhauer, Bonn & Guerra, 2019), it has resulted in an increase in incidences of vector-borne diseases such as Dengue fever and Malaria, especially in areas with poor planning and management practices (Knudsen & Slooff, 1992). Vector-borne diseases make up more than 17% of all infectious diseases and results in over one million deaths a year (World Health Organization [WHO], 2017). In particular, diseases spread by the *Aedes* spp. mosquitoes such as Dengue, Chikungunya and Zika pose a serious health risk in cities due to the mosquito's affinity towards urban areas (Koou et al., 2014). Urbanization inevitably results in more breeding sites for these mosquitoes as stagnant water sources increase due to improper waste disposal practices, open trash cans, and poor surface-water drainage (Lee et al., 2019).

Malaysia is on the list of countries that have high incidences of Dengue outbreaks, with Dengue cases gradually increasing over the years (European Centre for Disease Prevention and Control, 2019). With limited vaccines available to minimise the spread of vector-borne diseases, prevention and control continue to be the main mitigation strategies (Benelli, Jeffries & Walker, 2016; Fournet et al., 2018). For mosquito-borne diseases, there are three main approaches: 1) chemical control that involves fogging (i.e. insecticide spraying) to kill adult mosquitos (Usuga et al., 2019); 2) biological control that uses natural predators of mosquito larvae; and 3) environmental management and integrated vector management to reduce the mosquito breeding grounds (Amal et al., 2011). Of these methods, fogging is the most common form of adult mosquito population control in Malaysia, and is mainly carried out by both the Ministry of Health and the private sector in urban areas that experience vector-borne disease outbreaks (Amin et al., 2019).

Studies examining the efficiency of fogging in controlling adult mosquito populations have yielded mixed results. Some demonstrate short-lived effective mosquito population control (Amal et al., 2011), but others show evidence of mosquito populations developing increasing resistance towards commonly used fogging insecticides (Marcombe et al., 2011; Shafie, Tahir, &

Sabri, 2012). The long-term cost of mosquito developing resistance to insecticides outweighs the benefits of temporary reductions in adult populations, especially when new reports of Dengue regularly emerge in recently treated areas (Usuga et al., 2019).

A major source of concern for urban biodiversity is that sanctioned insecticides used in fogging are not explicitly selective towards mosquitoes - this poses a serious threat to non-target invertebrate communities that share the same habitats as mosquitoes (Braak et al., 2018). For example, studies have shown that natural insecticides such as pyrethrins can kill a wide range of insects but is ineffective in killing its targeted species – mosquitoes (Kwan et al., 2009; Abeyasuriya et al., 2017). As such, more studies are needed to understand how fogging affects non-target invertebrates in the urban environment.

Here, we investigate the impact of mosquito fogging on: 1) its target invertebrate taxon (Diptera); 2) selected non-target invertebrate taxa; and 3) the foraging behavior of an invertebrate pollinator taxon (Lepidoptera) within an urban tropical forest.

Materials & Methods

Study area

Our study was conducted in Kota Damansara Community Forest (KDCF) (3.17°N, 101.58°E), a secondary forest located in Selangor, one of the most urbanized states in Malaysia (Yaakob, Masron & Masami, 2012). The forest is under the management of Forestry Department Malaysia (permit number for this experiment: PHD.ST.052/2019) and has diverse invertebrate community. Over 13 different insect orders, mainly Coleoptera, Hymenoptera and Diptera were collected in a previous study (Khadijah, Azizah & Meor, 2013). As of September 2019, Selangor was the state with the highest reported cases of Dengue and Chikngunya disease in Malaysia (European Centre for Disease Prevention and Control, 2019). While the interior of KDCF is not fogged, its surrounding suburban areas are constantly fogged, making KDCF an ideal study site to examine the indirect effect of fogging on urban invertebrate (Fig. 1).

Figure 1. The study site – Kota Damansara Community Forest Reserve (KDCF)

The entrance to KDCF (filled circle) is surrounded by a government school – SMK Seksyen 10 Kota Damansara (dark grey square), the high rise condominium – De Rozelle (dark grey triangle). KDCF experiences regular fogging by different private companies in an effort to control vector-borne mosquito diseases (Kota Damansara residents, 2019, pers. comm.). Image credit: OpenClipart at <https://freesvg.org/>.

Ten trees within the KDCF were chosen for fogging treatments (Supplementary Table 1). The criteria for these trees are: (1) each tree is at least 100 m away from each other to prevent fogging overlap; (2) each tree is within the height range of 3 m for standardized vertical fogging dispersion; (3) each tree has an umbrella-like canopy cover with less than 10% herbivory damage on the canopy leaves for standardized horizontal surface area exposed to fogging; and (4) each tree is within 1 km away from the hiking trail as mosquitos tend so seek human hosts around hiking trails.

Fogging treatments

Insecticide fogging was carried out twice a week at 1100 h for a total of five weeks in the months of August to September 2019. The fogging time for mosquito control should ideally be around dawn or dusk for most effective mosquito control (Amal et al. 2011). However, for the purpose of our experiment, we choose 1100 h as these are the times where most hikers are not using the KDCF trails and pollinators are most active. Nevertheless, we assumed that mosquitoes would be present in the canopy regardless of our fogging time based on a recent study conducted in KDCF (Lee et al., 2019). Professional fogging personnel from Ridpest Sdn Bhd (<https://www.ridpest.com/>) were employed to carry out the fogging experiment using a hand held pulse thermal fog generator (Fig. 2a). The Detral 2.5 EC insecticide brand, which consisted of the active ingredient deltamethrin 2.5% w/w, was utilized for the fogging treatment (Fig. 2b). Deltamethrin is a synthetic pyrethroid commonly used for mosquito fogging that targets the nervous system of invertebrates (Chrutek et al., 2018). This insecticide solution was prepared to the specified dosage (1:200 ratio of insecticide to water) according to instructions on the bottle label, used for normal fogging around residential areas. The licensed foggers would fog the tree

starting at the bottom, thus allowing the fog to disperse to the top of the canopy (Fig. 2a). Each tree was fogged for 10 minutes, which is the minimum standard duration set by the Ministry of Health (<http://www.moh.gov.my/>). The standard duration for fogging is between 10 to 15 min depending on the severity of the mosquito-borne diseases reported and land area intended to be covered. For this experiment, the lower bound of the fogging time range as well as the insecticide used were chosen to simulate effects of conventional fogging practices for mosquitoes. KDCF itself is not normally directly fogged, but the study sites are likely to experience spill over effects of fogging and thus are ideal to investigate the indirect effects of fogging (Fig. 1). Nevertheless, mosquito populations remain relatively high in the KDCF, as hikers often spray insecticide on their exposed body parts to ward off mosquito bites (Wong EL, 2019, pers. comm.).

Figure 2. Fogging experiments set-up and example of invertebrate collected.

(A) Licensed foggers using hand-held pulse thermal fog generators to fog one of the study site. (B) The fogging chemical Detral 2.5 EC brand used for in this study. The active chemical (deltamethrin 2.5% w/w) is a form of synthetic Pyrethroid, claimed to be an effective insecticide targeting houseflies and mosquitoes. (C) Two 2.5 m and two 1.25 m polyethylene sheets set-up under the tree to fully cover the canopy of the site to maximize capture of knockdown invertebrate from the site. The sheets are held off the forest floor using 70 cm stakes to prevent leaf-litter invertebrates from crawling onto the sheets. (D) An example of dead soft-bodied invertebrate (order Araneae) due to fogging insecticide.

To collect knocked down invertebrates, two 2.5 m and two 1.25 m white polyethylene sheets were set up under the tree corresponding approximately to the canopy cover (Fig. 2c). The sheets were held up off the forest floor by 70 cm stakes to prevent the leaf-litter invertebrates from crawling onto the polyethylene sheets. Invertebrates knocked down by the fog onto the sheets were carefully collected five minutes post fogging treatment into plastic containers that were covered with small nets for ventilation. Collected invertebrates were brought back to the lab for classification and sorting.

177

178 Impact of fogging on target and non-target invertebrate taxa

179 To assess whether fogging was effective in killing its target invertebrate taxon (Diptera), and the
 180 extent to which it was detrimental to non-target invertebrate orders, we recorded their mortality
 181 3-hr after the fogging treatment. This time frame was chosen to understand the short-term effects
 182 of fogging on non-target invertebrate mortality rates. Invertebrates that responded to a light touch
 183 stimulus were categorised as ‘alive’ and those that remained motionless as ‘dead’. Each
 184 invertebrate was then sorted into their respective orders based on their morphological
 185 characteristics with appropriate taxonomic keys (McGavin, 1990; Imes, 1992).

186

187 Impact of fogging on foraging behaviour of an invertebrate pollinator

188 To determine whether fogging was detrimental on the foraging behaviour of invertebrate
 189 pollinators, we selected Lepidoptera as the focal taxon as they are important tropical pollinators,
 190 easily recognizable and also play a vital role as environmental indicators (Tzortzakaki et al.,
 191 2019). We quantified the number of butterflies occurring at each of the 10 sites pre- and post-
 192 fogging. On each day of the fogging treatment, the number of butterflies recorded in the 500 m
 193 radius of the site was recorded by two observers, each responsible for half of the radius. The
 194 counting of butterflies was conducted for 30 min pre-fogging treatment. For post-fogging count,
 195 the same observation radius was repeated with the same observers, at the same site and time
 196 (approximately 1000 h) for the same duration of time (30 min) 24 hours after fogging treatment.

197

198 Statistical analysis

199 We analysed the data collected from 10 sites in a Bayesian framework to quantify comparisons
 200 of group means: 1) number of alive vs. dead individuals of Diptera 3 hr post-fogging; 2) number
 201 of alive vs. dead individuals of selected invertebrate taxa 3 hr post-fogging (selected based on
 202 detections in at least 8 of 10 sites); and 3) number of Lepidoptera pre- vs. post-fogging. We used
 203 a Bayesian framework as it allows for relaxation of assumptions that t-tests require (Szucs &
 204 Ioannidis, 2017). We conducted a BEST analysis (Bayesian Estimation Supersedes the t-Test;

Kruschke, 2013), an alternative to t-tests that produces posterior estimates for group means. While BEST has been mainly utilised in psychological studies, it has already been used in ecological studies (e.g. Goh & Hashim, 2019). All analysis was conducted in R ver. 3.5.3 using package BEST (Kruschke & Meredith, 2018).

Results

A total of 1874 invertebrates were collected from 19 different orders after the 3-hr post fogging treatments. An ‘Unknown’ order consisting of 13 individuals could not be identified based on its morphological characteristics. These individuals are mostly immature form of some invertebrates (Table 1). Of the total number of invertebrates collected, 72.7% (1363) were knocked down by fogging and considered ‘dead’, with Diptera (8.8% of total knockdown insects) being the third most abundant order recorded as ‘dead’ (Table 1). Out of all the Diptera individuals knocked down, no mosquitoes were collected, despite their presence verified by field researchers who were bitten by them during fogging experiments.

Table 1:
Summary statistics of knocked-down invertebrate taxa after the 3-hr post fogging treatment across 10 sites in Kota Damansara Community Forest (KDCF), Selangor, Peninsular Malaysia.

The table is ordered from the most abundant to the least abundant knocked down invertebrate orders. Soft bodied invertebrate orders are indicated with an asterisk.

Impact of fogging on target invertebrate taxon

There were credible differences between the number of alive vs. dead Diptera individuals 3 hr post-fogging, with a larger number of Diptera recorded dead after fogging (mean difference = 12.7 individuals \pm SE 3.90 individuals); this can be seen by the lack of overlap between the Highest Density Intervals (HDIs) of $\mu_1 - \mu_2$ and the value of zero (Fig. 3).

Figure 3. Graphs representing the abundance of ‘Dead’ and ‘Alive’ Diptera post-fogging treatment across 10 sample sites.

(A) The Bayesian highest density interval (HDI) distribution of the difference between the number of “Dead” vs. “Alive” Diptera 3-hrs post-fogging. The lack of overlap between the Highest Density Intervals (HDIs) of $\mu_1 - \mu_2$ and the value of zero indicates a credible difference between the means. (B) A violin plot representing the distribution of “Dead” and “Alive” Diptera individuals found across the 10 sample sites. The distributions indicate that there are less “Alive” Diptera 3-hrs post-fogging. This can be seen in the larger distribution observed at the lower values of the “Alive” violin plot.

Impact of fogging on selected non-target invertebrate taxa

Invertebrate orders can also be classified into ‘soft-bodied’ and ‘hard-bodied’ based on their level of chitinization, i.e. the degree of chitin they possess in their exoskeletons. Only 9 invertebrate orders (excluding Diptera) were selected as they had detections in at least 8 of the sample sites. Among these, we found credible differences between the number of alive vs. dead individuals for 3 orders: Araneae (Fig. 2d), Collembola and Psocoptera, all of which are soft-bodied (Supplementary Figure 1).

Impact of fogging on foraging behaviour of an invertebrate pollinator

There were credible differences between the number of Lepidoptera observed pre- and post fogging, with a larger number of Lepidoptera recorded prior to fogging (5.2 individuals \pm SE 2.5 individuals); this can be seen by the lack of overlap between the Highest Density Intervals (HDIs) of $\mu_1 - \mu_2$ and the value of zero as well as the violin plots of the data distribution (Fig. 4). Given the data and priors, there is a 98% probability that the mean number of Lepidoptera decreased after fogging.

Figure 4. Graphs representing the number of Lepidoptera observations before and after (24 hours) fogging treatment at 10 sample sites.

(A) The Bayesian highest density interval (HDI) distribution of the difference between the number of Lepidoptera observations “Before” and “After” fogging. Given the data and priors, there is a 98% probability that the mean number of Lepidoptera decreased after fogging. (B) A violin plot representing the distribution of Lepidoptera observations “Before” and “After” fogging across the 10 sample sites. The distributions indicate that there are less Lepidoptera observations 24 hours post-fogging treatment. This is observed where the distribution of data is larger at the lower values of the “After” violin plot.

Discussion

To our knowledge, this is the first study to demonstrate short-term detrimental effects of mosquito fogging on urban invertebrates in a tropical city in Southeast Asia. Our results demonstrate that the fogging insecticide had an unintended adverse effect on non-target invertebrates, which is characterized in this study as negative effects on invertebrates that were not mosquitoes. Similar results were also observed by Abeyasuriya et al. (2017) in Sri Lanka, where more dead than alive individuals were recorded amongst the 12 insect orders sampled post- 24-hr fogging.

Our findings are, however, not concordant with previous studies that found that Diptera was among the most affected by fogging (Kwan et al., 2009; Abeyasuriya et al., 2017). In our results, Hymenoptera (consisting of ants, bees and wasps) was the most affected by fogging (Table 1). One possible explanation could be sites from both Abeyasuriya et al. (2017) and Kwan et al. (2009) studies had very different target and non-target invertebrate compositions, which are very dependent on the floral composition and the niches available at each site (Toft et al., 2019). At our study sites, the floral composition is of natural secondary forest composition, whereas Abeyasuriya et al. (2017) and Kwan et al. (2009) studies focus on cultivated landscape. Previous studies at KDCF have sampled mosquitoes such as *Aedes aegypti* and *Culex* spp. residing and foraging around the vegetation (Khadijah, Azizah & Meor, 2013; Lee et al., 2019). Therefore, the absence of mosquito species in our experiment suggests that mosquitoes species at our

study have adapted to frequent fogging in the area, either by developing resistance towards the Deltamethrin (Demok et al., 2019) or by developing mechanisms to evade its influence.

The unintended effect of fogging on non-target invertebrates is alarming as many of them play vital functions in urban ecosystems. Thysanoptera, for example, encompassing 11% of the total knocked down samples, was the sixth most affected order with 76.4% ‘dead’ 3-hr post-fogging. Commonly known as thrips, these invertebrates are important pollinators for many Dipterocarpaceae, an important hard-wood tree family that make up Southeast Asia’s rainforest tree communities (Apanah & Chan, 1981). Thrips are also pollinators of *Macaranga* species (Fiala et al., 2011), an important pioneer tree genus for forest regeneration in Malaysia (Daisuke et al., 2013). An adverse effect on thrips diversity and numbers could severely disrupt pollination cycles of these two very important tree families, affecting existing dipterocarp tree biodiversity and might impede any forest restoration projects that plants *Macaranga* species.

Our study also reflects the varying degrees of insecticide susceptibility in invertebrates. Insecticide penetration may be less efficient in invertebrates with thicker cuticles and thus decrease their susceptibility to insecticides (Dang et al., 2017). Our results show that fogging mainly affected small, soft-bodied invertebrates such as Hemiptera (commonly known as true bugs), Collembola (commonly known as springtails) and Psocoptera (commonly known as booklice), consistent with other studies (Boyce et al., 2007; Abeyasuriya et al., 2017). Fogging may affect soft-bodied invertebrates more due to their relatively lower levels of chitinization. Reduced chitinization in the cuticles of soft-bodied invertebrates may permit easier entry of pyrethroids such as deltamethrin into the body as they primarily enter the body through skin contact and hence persist longer (Chrutek et al., 2018). In contrast, Coleoptera, which have unique adaptations of hardened forewings and compact bodies with very high-level of chitinization (McGavin, 1990; Imes, 1992) are more resistant to fogging (Table 1). In our study only 42.7% of Coleoptera died from fogging where majority survived. Our results are consistent with a study by Abeyasuriya et al. (2017) where insects belonging to the order Coleoptera had the lowest mortality rate in two out of their three study sites. Even though hardened adult Coleoptera are more resistant to fogging insecticides, its larvae stages could still be affected.

Our findings indicate that fogging also has negative impacts on invertebrate pollinators such as butterflies. Sublethal exposure to insecticide can lead to changes in Lepidoptera foraging

behavior as the insecticides can alter the odor emitted by the plant (de Franca et al., 2017). This could be due to butterflies avoiding the insecticides that attach onto pollen and nectar (van der Sluijs et al., 2013) or the fog has not dispersed completely under the dense canopy. Future studies can focus on counting the number of Lepidoptera individuals in the fogged area for a longer period to investigate the extent they can recover to pre-fogging conditions. This result could give an indication of the length the fog persists on the surrounding vegetation. As our study only examined short-term effects of fogging on Lepidoptera, it is still unclear whether fogging has any long-term effects on pollinator behavior or physiology. Studies indicate that insecticides that target the nervous systems of invertebrates reduce pollinator survival and reproduction rates (Abeyasuriya et al., 2017; de Franca et al., 2017). While immediate fogging may not directly affect pollinators such as butterflies and bees, these organisms may become exposed to these chemicals through feeding and foraging (Braak et al., 2018) as pyrethroids have been shown to stick to pollen (Pettis et al., 2013). As evidenced from our study, most Lepidoptera individuals that were affected by fogging were caterpillars feeding on the vegetation when the fog hit.

In general, there is still a paucity of information on threats to invertebrate communities in urban areas. Most urban ecology studies have focused on the consequences of pollinator species decline (Thogmartin et al., 2017; Meeus et al., 2018; Wepprich et al., 2019), but very few studies have examined the consequences of general invertebrate decline. One possible consequence of the decline in non-target invertebrates is a negative effect on the survival of insectivorous birds, frogs, lizards and other invertebrate predators (spiders, wasps etc) that rely on invertebrates in their diet (Sanchez-Bayo & Wyckhuys, 2019). While fogging may not kill all invertebrates, the sub-lethal dosage exposed to these invertebrates may also have possible consequences on their biology, physiology and behavior (de Franca et al., 2017). Fogging may also lead to the homogenization of invertebrate species with generalist dominating the remnant habitat, reducing diversity and disrupting invaluable ecosystem services such as pollination, decomposition and nutrient cycling (Sanchez-Bayo & Wyckhuys, 2019).

Caveats

Our results could have been more robust if we had adopted a Before-After-Control-Impact (BACI) design, but limited resources were a constraint. We also acknowledge that our results were only reflective for the number of knocked-down insects that had dropped onto the collection sheets - they do not take into account the number of invertebrates, unaffected or affected, which remained in the canopy post-fogging. Future studies could account for this bias by sampling the canopy level and hidden crevices and leaves for a better representation of unaffected and affected invertebrates. Furthermore, as this study examines the short-term effects of fogging on non-target invertebrates, the cut-off timing for 'Dead' or 'Alive' categorization should be extended in future studies. This is to ensure that long-term effects can be captured by recording the number of invertebrates, initially recorded as 'Alive' that eventually succumbed. Abeyasuriya *et al.* (2017) used a 24 hr window as their cut-off point, and future study could benefit by mirroring this 24 hr period. While our study has documented invertebrates that are adversely affected by fogging, it would have been ideal to identify invertebrates to morphospecies to accurately determine differences in species diversity and richness due to fogging. However, many of the invertebrates collected were relatively small and of immature developmental stages where identification keys were absent. Metabarcoding could be explored in the future to obtain more accurate representation of species diversity. By doing so, the ecological functional groups of the invertebrates affected by fogging can also be identified.

Conclusion

Overall, our study shows that insecticide fogging can be detrimental to non-target invertebrates, particularly pollinators and soft-bodied species. Alternative methods of mosquito control should be explored in order to reduce health risks in tropical cities, while preserving other forms of urban biodiversity.

Acknowledgements

We thank Ms. Harlina Binti Md Yunus and Forestry Department Malaysia for permission and logistics assistance. The fogging experiment could not run smoothly without the help from Ms

Deniece Yeo Yin Chia, Ms Taneswarry Sethu Pathy and Mr Jason Yew Seng Gan that assist in fogging experiments and subsequent invertebrate sorting. We also thank Ms Wong Ee Lyn and Kota Damansara Community Forest (KDCF) regular hikers for inviting us to conduct this experiment at their managed forest to understand the effect of fogging on urban biodiversity

References

- Abeyasuriya KG**TN, **Nugapola N**WNP, **Perera M**DB, **Karunaratne W**AIP, **Karunaratne SH**PP. 2017. Effect of dengue mosquito control insecticide thermal fogging on non-target insects. *International Journal of Tropical Insect Science* **37**(1):11-18. DOI: 10.1017/S1742758416000254
- Amal A**R, **Malina O**, **Rukman A**H, **Zasmy U**N, **Omar A**W, **Norhafizah M**. 2011. The impact of preventive fogging on entomological parameters in a university campus in Malaysia. *Malaysian Journal of Medicine and Health Sciences* **7**(1):9-15.
- Amin L**, **Arham A**F, **Mahadi Z**, **Razman M**R, **Rusly N**S. 2019. Stakeholder's attitude towards fogging technique in Malaysia. *Akademika* **89**(2):187-200. DOI: <https://doi.org/10.17576/akad-2019-8902-14>
- Apanah S**, **Chan H**T. 1981. Thrips: the pollinators of some dipterocarps. *Malaysian Forester* **44**(2-3):234-252.
- Benelli G**, **Jeffries C**L, **Walker T**. 2016. Biological control of mosquito vectors: Past, present, and future. *Insects* **7**(4):52. DOI: 10.3390/insects7040052
- Boyce W**M, **Lawler S**P, **Schultz J**M, **McCauley S**J, **Kimsey L**S, **Niemela M**K, **Nielsen C**F, **Reisen W**K. 2007. Nontarget effects of the mosquito adulticide pyrethrin applied aurally during a West Nile virus outbreak in an urban California environment. *Journal of the American Mosquito Control Association* **23**(3):335-9. DOI: 10.2987/8756-971X(2007)23[335:NEOTMA]2.0.CO;2

400 **Braak N, Neve R, Jones AK, Gibbs M, Brueker J. 2018.** The effects of insecticides on
 401 butterflies – A review. *Environmental Pollution* **242**:507-518. DOI:
 402 <https://doi.org/10.1016/j.envpol.2018.06.100>

403 **Chrustek A, Holyńska-Iwan I, Dziembowska I, Bogusiewicz J, Wróblewski M, Cwynar A,**
 404 **Olszewska-Slonina D. 2018.** Current research on the safety of pyrethroids used as insecticides.
 405 *Medicina (Kaunas)* **54**(4):61. DOI: 10.3390/medicina54040061

406 **Clark PJ, Reed JM, Chew FS. 2007.** Effects of urbanization on butterfly species richness, guild
 407 structure, and rarity. *Urban Ecosystem* **10**:321-337. DOI: 10.1007/s11252-007-0029-4

408 **Daisuke H, Tanaka K, Jawa KJ, Ikuo N, Katsutoshi S. 2013.** Rehabilitation of degraded
 409 tropical rainforest using dipterocarp trees in Sarawak, Malaysia. *International Journal of*
 410 *Forestry Research* **2013**(2013):1-11.

411 **Dang K, Doggett, SL, Singham GV, Lee, C-Y. 2017.** Insecticide resistance and resistance
 412 mechanisms in bed bugs, *Cimex* spp. (Hemiptera: Cimicidae). *Parasites and Vectors* **10**(318).
 413 DOI 10.1186/s13071-017-2232-3

414 **de França SM, Breda MO, Barbosa DRS, Araujo AMN, Guedes CA. 2017.** The sublethal
 415 effects of insecticides in insects. In: Shields VDC, ed.. *Biological Control of Pest and Vector*
 416 *Insects*. London: IntechOpen 23-39.

417 **Demok S, Endersby-Harshman N, Vinit R, Timinao L, Robinson LJ, Susapu M, Makita L,**
 418 **Laman M, Hoffman A, Karl, K. 2019.** Insecticide resistance status of *Aedes aegypti* and *Aedes*
 419 *albopictus* mosquitoes in Papua New Guinea. *Parasites and Vectors* **12**(1):333. DOI:
 420 <https://doi.org/10.1186/s13071-019-3585-6>

421 **Eisenhauer N, Bonn A, Guerra CA. 2019.** Recognizing the quiet extinction of invertebrates.
 422 *Nature Communications* **10**(1):50. DOI: <https://doi.org/10.1038/s41467-018-07916-1>

423 **European Centre for Disease Prevention and Control. 2019, 19 October.** Communicable
 424 disease threats report. Retrieved from [https://www.ecdc.europa.eu/en/publications-](https://www.ecdc.europa.eu/en/publications-data/communicable-disease-threats-report-13-19-october-2019-week-42)
 425 [data/communicable-disease-threats-report-13-19-october-2019-week-42](https://www.ecdc.europa.eu/en/publications-data/communicable-disease-threats-report-13-19-october-2019-week-42)

426 **Fiala B, Ute M, Hasim R, Maschwitz U. 2011.** Pollination systems in pioneer trees of the genus
 427 Macaranga (Euphorbiaceae) in Malaysian rainforests. *Biological Journal of the Linnean Society*
 428 **103**(4):935-953. DOI: 10.1111/j.1095-8312.2011.01680.x

429 **Ferreira R, Martins P, Dutra C, Mentone M, Antonini, B. 2013.** Old fragments of forest
 430 inside an urban area are able to keep orchid bee (Hymenoptera: Apidae: Euglossini)
 431 assemblages? The case of a Brazilian historical city. *Neotropical Entomology* **42**(5): 466–473.
 432 DOI: <https://doi.org/10.1007/s13744-013-0145-1>

433 **Forman RT. 2014.** *Urban ecology: science of cities*. Cambridge University Press.

434 **Fournet F, Jourdain F, Bonnet E, Degroote S, Ridde V. 2018.** Effective surveillance systems
 435 for vector-borne diseases in urban settings and translation of the data into action: a scoping
 436 review. *Infectious Diseases of Poverty* **7**(1):99. DOI: <https://doi.org/10.1186/s40249-018-0473-9>

437 **Goh TG, Hashim R. 2019.** Trait responses of Peninsular Malaysian dung beetles (Scarabaeidae:
 438 Scarabaeinae) to the loss of megafauna dung. *Journal of Tropical Ecology* 1-3

439 **Hennig EI, Ghazoul J. 2011.** Pollinating animals in the urban environment. *Urban Ecosystems*
 440 **15**(1):149-166. DOI: 10.1007/s11252-011-0202-7

441 **Hülsmann M, von Wehrden H, Klein A-M, Leonhardt SD. 2015.** Plant diversity and
 442 composition compensate for negative effects of urbanization on foraging bumble bees.
 443 *Apidologie* **46**:760–770. DOI: <https://doi.org/10.1007/s13592-015-0366-x>

444 **Imes R. 1992.** *The practical entomologist*. New York, N.Y: Simon and Schuster.

445 **Khadijah AR, Azizah AA, Meor SR. 2013.** Diversity and abundance of insect species at Kota
 446 Damansara Community Forest Reserve, Selangor. *Scientific Research and Essays* **8**(9):359-374.
 447 DOI 10.5897/SRE12.481

448 **Knudsen AB, Slooff R. 1992.** Vector-borne disease problems in rapid urbanization: new
 449 approaches to vector control. *Bulletin of the World Health Organization* **70**(1):1-6.

450 **Kruschke JK. 2013.** Bayesian Estimation Supersedes the t-Test. *Journal of Experimental*
 451 *Psychology: General* **142**:573–603.

452

453 **Kruschke JK, Meredith M. 2018.** BEST: Bayesian Estimation Supersedes the t-Test. R
454 package ver. 0.5.1. 2018. Available at <https://cran.r-project.org/web/packages/BEST>.

455 **Koou S-Y, Chong C-S, Vythilingam I, Ng L-C, Lee C-Y. 2014.** Pyrethroid resistance in *Aedes*
456 *aegypti* larvae (Diptera: Culicidae) from Singapore. *Journal of Medical Entomology* **51**(1):170-
457 181. DOI: <https://doi.org/10.1603/ME13113>

458 **Kwan JA, Novak MG, Hyles TS, Niemela MK. 2009.** Mortality of nontarget arthropods from
459 an aerial application of pyrethrins. *Journal of the American Mosquito Control Association*
460 **25**(2):218-20. DOI: 10.2987/08-5858.1

461 **Lee JM, Wasserman RJ, Gan JY, Wilson RF, Rahman S, Yek, SH. 2019.** Human activities
462 attract harmful mosquitoes in a tropical urban landscape. *EcoHealth* **17**(1):52-63. DOI
463 10.1007/s10393-019-01457-9

464 **MacGregor CJ, Pocock MJO, Fox R, Evans DM. 2015.** Pollination by nocturnal Lepidoptera,
465 and the effects of light pollution: a review. *Ecological Entomology* **40**(3):187-198. DOI:
466 10.1111/een.12174

467 **Marcombe S, Darriet F, Tolosa M, Agnew P, Duchon S, Etienne M, Yp Tcha MM,**
468 **Chandre F, Corbel V, Yébakima A. 2011.** Pyrethroid resistance reduces the efficacy of space
469 sprays for dengue control on the island of Martinique (Caribbean). *PLoS Neglected Tropical*
470 *Diseases* **5**(6):e1202. DOI: 10.1371/journal.pntd.0001202

471 **McGavin GC. 1990.** *Insects, spiders and other terrestrial arthropods*. New York, NY: Dorling
472 Kindersley Inc.

473 **Meeus I, Pisman M, Smagghe G, Piot N. 2018.** Interaction effects of different drivers of wild
474 bee decline and their influence on host–pathogen dynamics. *Current Opinion in Insect Science*
475 **26**:136-141

476 **Pettis JS, Lichtenberg EM, Andree M, Stitzinger J, Rose R, Vanengelsdorp D. 2013.** Crop
477 pollination exposes honey bees to pesticides which alters their susceptibility to the gut pathogen
478 *Nosema ceranae*. *PLoS ONE* **8**(7):e70182.

479 **Rollings R, Goulson D. 2019.** Quantifying the attractiveness of garden flowers for
480 pollinators. *Journal of Insect Conservation* **23**(5-6):803-817.

481 **Sánchez-Bayo F, Wyckhuys KAG. 2019.** Worldwide decline of the entomofauna: A review of
482 its drivers. *Biological Conservation*, **232**:8-27. DOI:
483 <https://doi.org/10.1016/j.biocon.2019.01.020>

484 **Shafie FA, Tahir MPM, Sabri NM. 2012.** Aedes mosquitoes resistance in urban community
485 setting. *Procedia – Social and Behavioral Sciences* **36**:70-76. DOI:
486 [10.1016/j.sbspro.2012.03.008](https://doi.org/10.1016/j.sbspro.2012.03.008)

487 **Szucs, D, Ioannidis J. 2017.** When null hypothesis significance testing is unsuitable for
488 research: a reassessment. *Frontiers in Human Neuroscience* **11**:390.

489 **Thogmartin WE, Wiederholt R, Oberhauser K, Drum RG, Diffendorfer JE, Altizer S,**
490 **Taylor OR, Pleasants J, Semmens D, Semmens B, Erickson R, Kaitlin L, Lopez-Hoffman**
491 **L. 2017.** Monarch butterfly population decline in North America: identifying the threatening
492 processes. *Royal Society Open Science* **4**(9): 170760.

493 **Toft RJ, Ford DE, Sullivan JJ, Stewart GH. 2019.** Invertebrates of an urban old growth forest
494 are different from forest restoration and garden communities. *New Zealand Journal of*
495 *Ecology* **43**(1):1-10.

496 **Tzortzakaki O, Kati V, Panitsa M, Tzanatos E, Giokas S. 2019.** Butterfly diversity along the
497 urbanization gradient in a densely-built Mediterranean city: Land cover is more decisive than
498 resources in structuring communities. *Landscape and Urban Planning* **183**: 79-87. DOI:
499 <https://doi.org/10.1016/j.landurbplan.2018.11.007>

500 **Usuga AF, Zuluaga-Idárraga LM, Alvarez N, Roho R, Henao E, Rúa-Urbe GL. 2019.**
501 Barriers that limit the implementation of thermal fogging for the control of dengue in Colombia:
502 a study of mixed methods. *BioMed Central Public Health* **19**(1): 1–10. DOI:
503 <https://doi.org/10.1186/s12889-019-7029-1>

van der Sluijs JP, Simon-Delso N, Goulson D, Maxim L, Bonmatin J-M, Belzunces LP. 2013. Neonicotinoids, bee disorders and the sustainability of pollinator services. *Current Opinion in Environmental Sustainability* **5**(3-4): 293–305.

Wepprich T, Adrion JR, Ries L, Wiedmann J, Haddad NM. 2019. Butterfly abundance declines over 20 years of systematic monitoring in Ohio, USA. *PLoS ONE* **14**(7): e0216270.

World Health Organization (WHO). 2017. Vector-borne disease. Available at <https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases> (accessed 8 October 2019).

Yaakob U, Masron T, Masami F. 2012. Ninety years of urbanization in Malaysia: A geographical investigation of its trends and characteristics. *Ritsumeikan Journal of Social Sciences and Humanities* 79-102.

Zhang XQ. 2016. The trends, promises and challenges of urbanisation in the world. *Habitat International* **54**(3): 241-252. DOI: <https://doi.org/10.1016/j.habitatint.2015.11.018>

SUPPLEMENTARY INFORMATION

Supplementary Table 1. GPS Coordinates of 10 sites at KDCF

Supplementary Table 2. The raw data of dead and alive invertebrates at 10 sites from fogging experiments

Supplementary Table 3. The raw data from butterfly observation 1-hr pre-fogging and 1-day post fogging

529 Supplementary Figure 1. Bayesian highest density interval (HDI) distributions of the difference
 530 between the number of live vs. dead for 9 selected non-target invertebrate orders 3 hr post-
 531 fogging. Orders with asterisk* have a credible difference between means due to the lack of
 532 overlap between the Highest Density Intervals (HDIs) of $\mu_1 - \mu_2$ and the value of zero. Orders in
 533 red are considered hard-bodied and those in green are soft-bodied.

534

Figure 1

The study site - Kota Damansara Community Forest Reserve (KDCF)

The entrance to KDCF (filled circle) is surrounded by a government school - SMK Seksyen 10 Kota Damansara (dark grey square), the high rise condominium - De Rozelle (dark grey triangle). KDCF experiences regular fogging by different private companies in an effort to control vector-borne mosquito diseases (Kota Damansara residents, 2019, pers. comm.).

Image credit: OpenClipart at <https://freessvg.org/>.

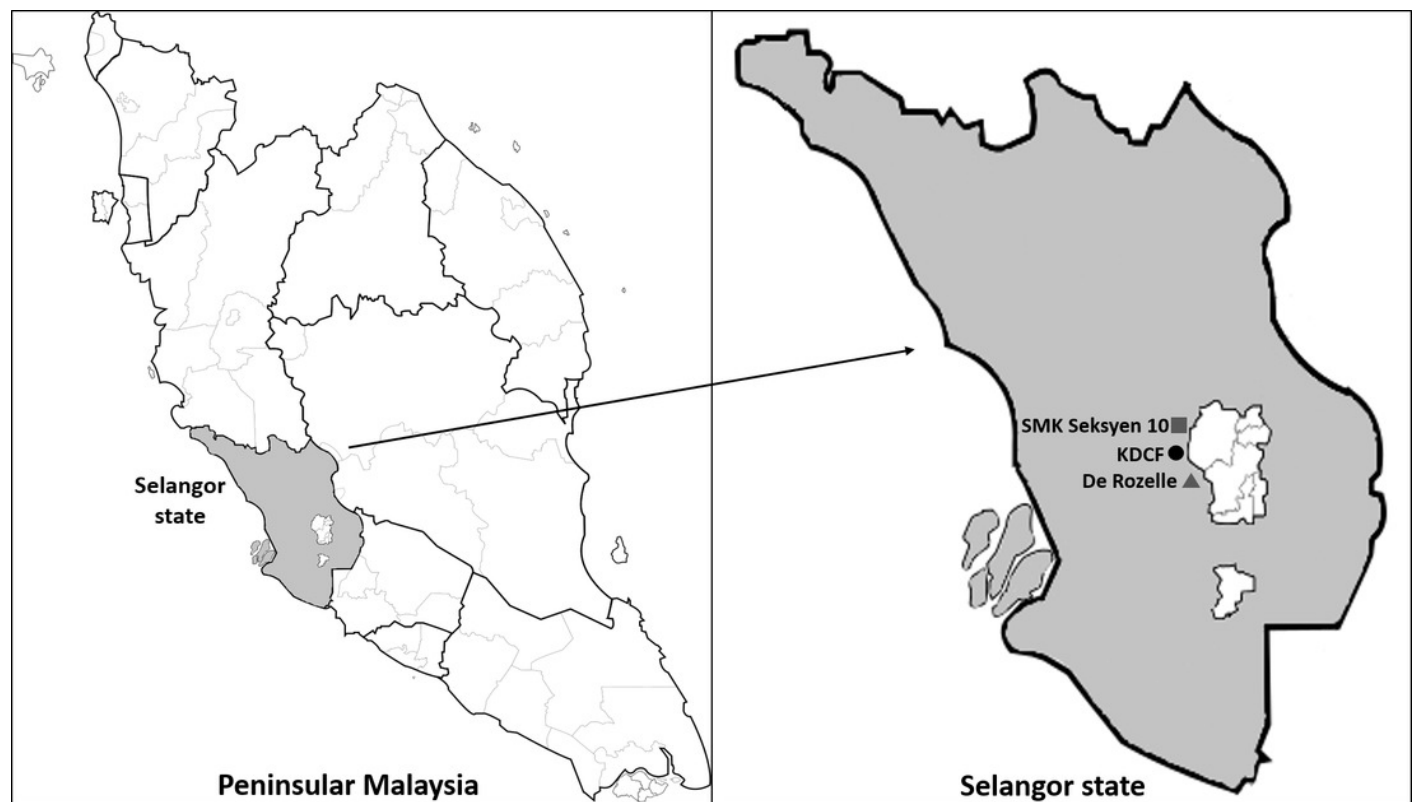


Figure 2

Fogging experiments set-up and example of invertebrate collected.

(A) Licensed foggers using hand-held pulse thermal fog generators to fog one of the study site. (B) The fogging chemical Detral 2.5 EC brand used for in this study. The active chemical (deltamethrin 2.5% w/w) is a form of synthetic Pyrethroid, claimed to be an effective insecticide targeting houseflies and mosquitoes. (C) Two 2.5 m and two 1.25 m polyethylene sheets set-up under the tree to fully cover the canopy of the site to maximize capture of knockdown invertebrate from the site. The sheets are held off the forest floor using 70 cm stakes to prevent leaf-litter invertebrates from crawling onto the sheets. (D) An example of dead soft-bodied invertebrate (order Araneae) due to fogging insecticide.

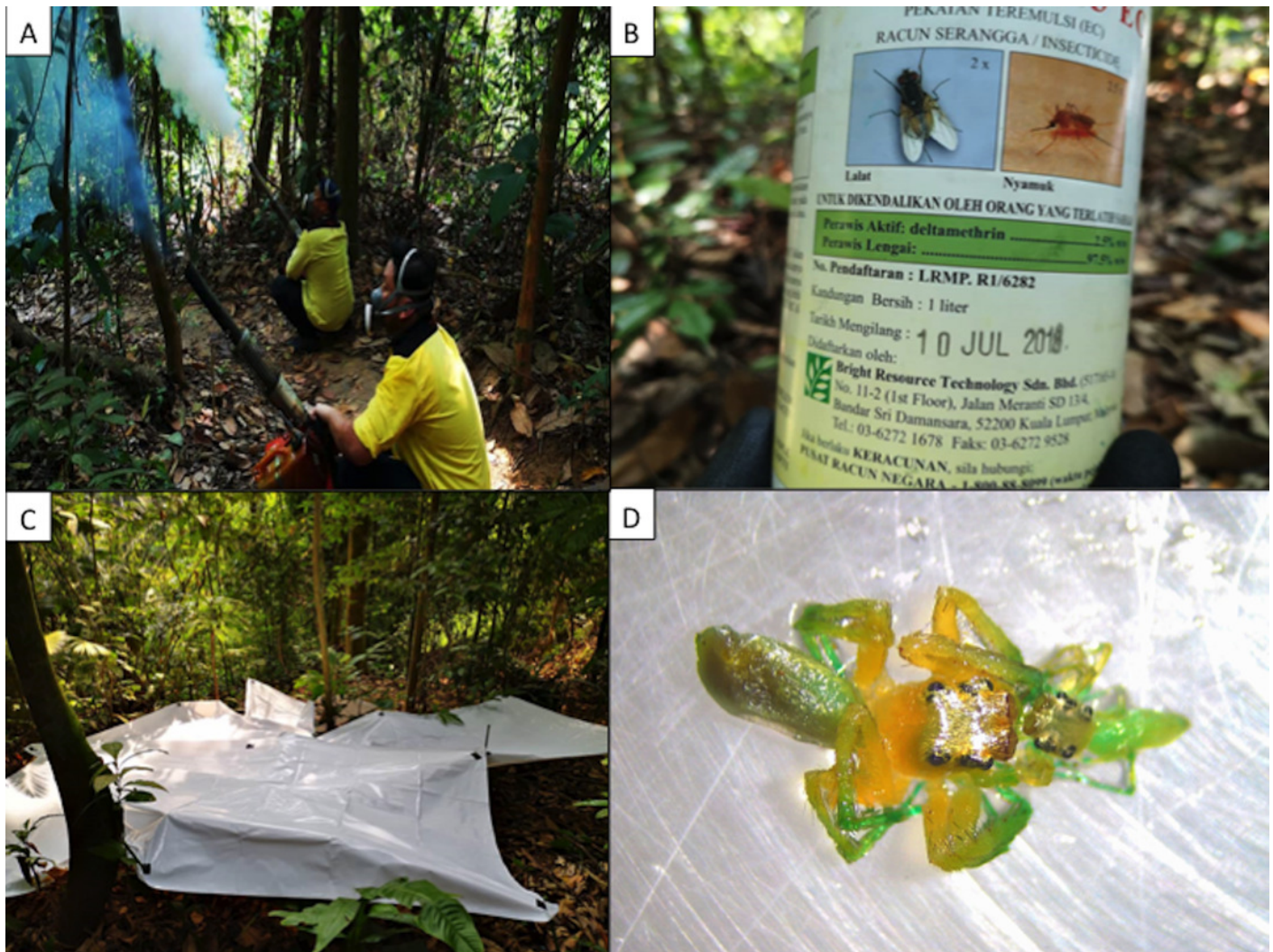


Table 1(on next page)

Summary statistics of knocked-down invertebrate taxa after the 3-hr post fogging treatment across 10 sites in Kota Damansara Community Forest (KDCF), Selangor, Peninsular Malaysia. .

The table is ordered from the most abundant to the least abundant knocked down invertebrate orders. Soft bodied invertebrate orders are indicated with an asterisk.

Order	Number of knocked down invertebrates	Dead	Alive	Mortality 3 hr post-fogging (%)
Hymenoptera	337	217	120	64.4
Araneae*	296	238	58	80.5
Hemiptera*	209	144	65	68.9
Thysanoptera*	208	159	49	76.4
Coleoptera	185	79	106	42.7
Diptera*	166	148	18	89.2
Collembola*	118	115	3	97.5
Psocoptera*	112	106	6	94.6
Acari*	63	47	16	74.6
Blattodea	51	38	13	74.5
Orthoptera*	57	33	24	57.9
Lepidoptera*	29	17	12	58.6
Pseudoscorpiones*	10	2	8	20.0
Archaeognatha*	5	4	1	80.0
Neuroptera*	5	3	2	60.0
Opiliones*	4	3	1	75.0
Phasmatodea	3	0	3	0.0
Diplopoda	2	1	1	50.0
Mantodea	1	0	1	0.0
Unknown	13	9	4	69.2
Total	1874	1363	511	72.7

1

Figure 3

Graphs representing the abundance of 'Dead' and 'Alive' Diptera post-fogging treatment across 10 sample sites.

(A) The Bayesian highest density interval (HDI) distribution of the difference between the number of "Dead" vs. "Alive" Diptera 3-hrs post-fogging. The lack of overlap between the Highest Density Intervals (HDIs) of $\mu_1 - \mu_2$ and the value of zero indicates a credible difference between the means. (B) A violin plot representing the distribution of "Dead" and "Alive" Diptera individuals found across the 10 sample sites. The distributions indicate that there are less "Alive" Diptera 3-hrs post-fogging. This can be seen in the larger distribution observed at the lower values of the "Alive" violin plot.

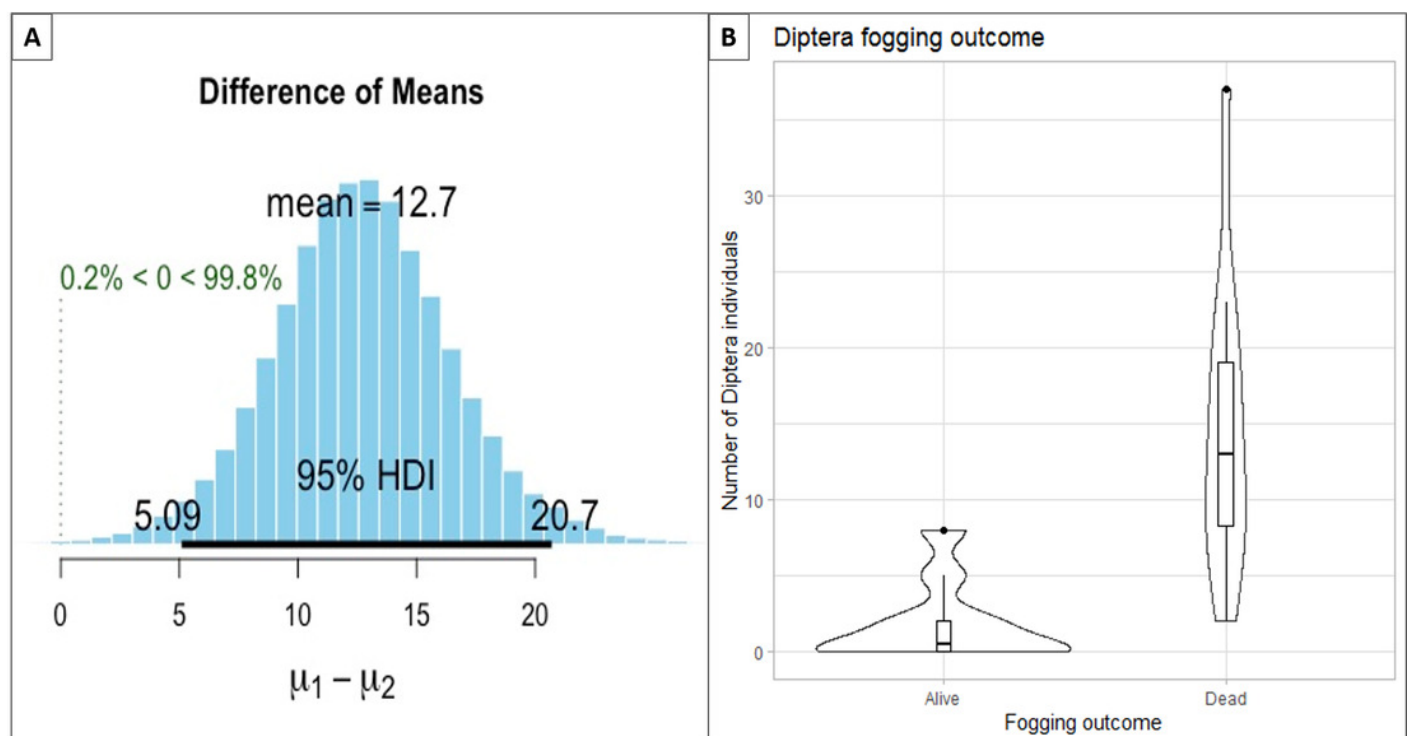


Figure 4

Graphs representing the number of Lepidoptera observations before and after (24 hours) fogging treatment at 10 sample sites.

(A) The Bayesian highest density interval (HDI) distribution of the difference between the number of Lepidoptera observations “Before” and “After” fogging. Given the data and priors, there is a 98% probability that the mean number of Lepidoptera decreased after fogging. (B) A violin plot representing the distribution Lepidoptera observations “Before” and “After” fogging across the 10 sample sites. The distributions indicate that there are less Lepidoptera observations 24 hours post-fogging treatment. This is observed where the distribution of data is larger at the lower values of the “After” violin plot.

