

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

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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.

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

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23 **Abstract**

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

35

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

43

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

52

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.

61 **Introduction**

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

75 Malaysia is on the list of countries that have high incidences of Dengue outbreaks, with
76 Dengue cases gradually increasing over the years (European Centre for Disease Prevention and
77 Control, 2019). With limited vaccines available to minimise the spread of vector-borne diseases,
78 prevention and control continue to be the main mitigation strategies (Benelli, Jeffries & Walker,
79 2016; Fournet et al., 2018). For mosquito-borne diseases, there are three main approaches: 1)
80 chemical control that involves fogging (i.e. insecticide spraying) to kill adult mosquitos (Usuga
81 et al., 2019); 2) biological control that uses natural predators of mosquito larvae; and 3)
82 environmental management and integrated vector management to reduce the mosquito breeding
83 grounds (Amal et al., 2011). Of these methods, fogging is the most common form of adult
84 mosquito population control in Malaysia, and is mainly carried out by both the Ministry of
85 Health and the private sector in urban areas that experience vector-borne disease outbreaks
86 (Amin et al., 2019).

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

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

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

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

104

105 **Materials & Methods**

106 **Study area**

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

117

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

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

124

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

132

133 **Fogging treatments**

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

148 starting at the bottom, thus allowing the fog to disperse to the top of the canopy (Fig. 2a). Each
149 tree was fogged for 10 minutes, which is the minimum standard duration set by the Ministry of
150 Health (<http://www.moh.gov.my/>). The standard duration for fogging is between 10 to 15 min
151 depending on the severity of the mosquito-borne diseases reported and land area intended to be
152 covered. For this experiment, the lower bound of the fogging time range as well as the
153 insecticide used were chosen to simulate effects of conventional fogging practices for
154 mosquitoes. KDCF itself is not normally directly fogged, but the study sites are likely to
155 experience spill over effects of fogging and thus are ideal to investigate the indirect effects of
156 fogging (Fig. 1). Nevertheless, mosquito populations remain relatively high in the KDCF, as
157 hikers often spray insecticide on their exposed body parts to ward off mosquito bites (Wong EL,
158 2019, pers. comm.).

159

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

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

169

170 To collect knocked down invertebrates, two 2.5 m and two 1.25 m white polyethylene sheets
171 were set up under the tree corresponding approximately to the canopy cover (Fig. 2c). The sheets
172 were held up off the forest floor by 70 cm stakes to prevent the leaf-litter invertebrates from
173 crawling onto the polyethylene sheets. Invertebrates knocked down by the fog onto the sheets
174 were carefully collected five minutes post fogging treatment into plastic containers that were
175 covered with small nets for ventilation. Collected invertebrates were brought back to the lab for
176 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;

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

209

210 **Results**

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

219

220 **Table 1:**

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

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

226

227 **Impact of fogging on target invertebrate taxon**

228 There were credible differences between the number of alive vs. dead Diptera individuals 3 hr
229 post-fogging, with a larger number of Diptera recorded dead after fogging (mean difference =
230 12.7 individuals \pm SE 3.90 individuals); this can be seen by the lack of overlap between the
231 Highest Density Intervals (HDIs) of μ_1 – μ_2 and the value of zero (Fig. 3).

232

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

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

242

243 **Impact of fogging on selected non-target invertebrate taxa**

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

250

251 **Impact of fogging on foraging behaviour of an invertebrate pollinator**

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

258

259 **Figure 4. Graphs representing the number of Lepidoptera observations before and after**
260 **(24 hours) fogging treatment at 10 sample sites.**

261 (A)The Bayesian highest density interval (HDI) distribution of the difference between the
262 number of Lepidoptera observations “Before” and “After” fogging. Given the data and priors,
263 there is a 98% probability that the mean number of Lepidoptera decreased after fogging.(B) A
264 violin plot representing the distribution Lepidoptera observations “Before” and “After” fogging
265 across the 10 sample sites. The distributions indicate that there are less Lepidoptera observations
266 24 hours post-fogging treatment. This is observed where the distribution of data is larger at the
267 lower values of the “After” violin plot.

268

269 **Discussion**

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

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

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

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

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

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

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

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

345

346 **Caveats**

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

365

366 **Conclusion**

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

371

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379

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517

518

519 **SUPPLEMENTARY INFORMATION**

520

521 Supplementary Table 1. GPS Coordinates of 10 sites at KDCF

522

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

525

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

528

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://freesvg.org/>.

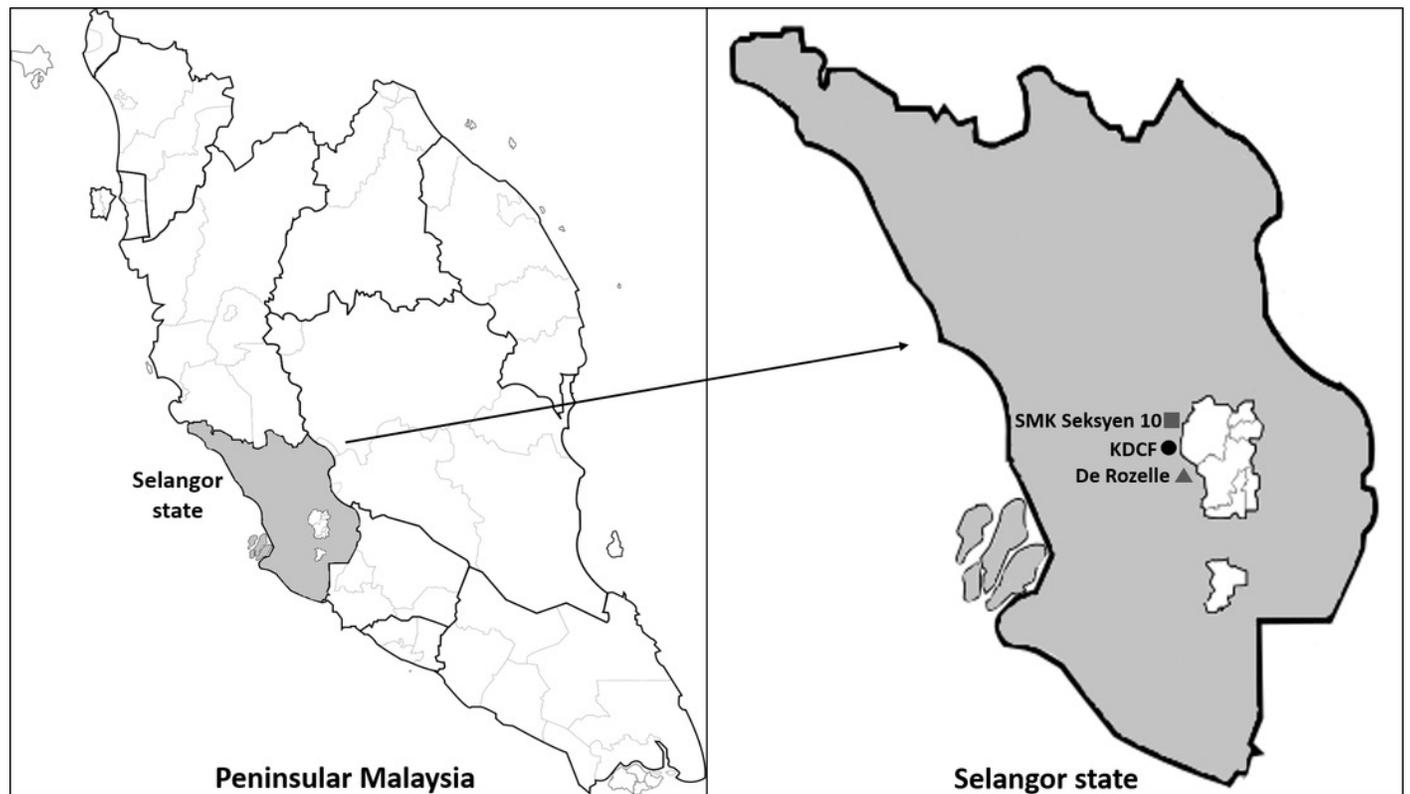


Figure 2

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.

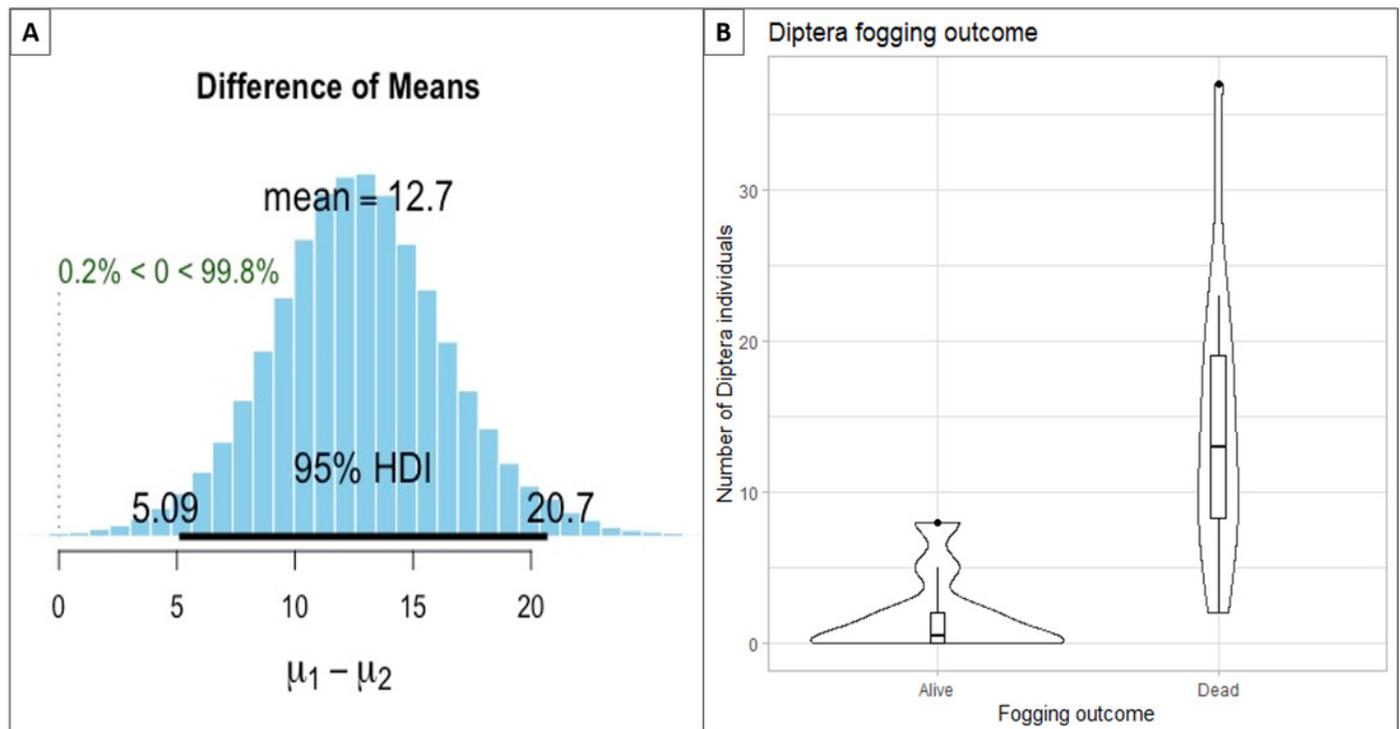


Figure 4

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.

