

## Original Article

# Employing Different Traps for Collection of Mosquitoes and Detection of Dengue, Chikungunya and Zika Vector, *Aedes albopictus*, in Borderline of Iran and Pakistan

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(Received 25 May 2020; accepted 06 Dec 2020)

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## Abstract

**Background:** Southeastern Iran has been established as an area with the potential to harbor Asian tiger mosquito populations. In 2013, a few numbers of *Aedes albopictus* were detected in three sampling sites of this region. This field study was aimed to evaluate the efficacy of various traps on monitoring mosquitoes and status of this dengue vector, in five urban and 15 suburban/rural areas.

**Methods:** For this purpose, four adult mosquito traps (BG-sentinel 2, bednet, Malaise, and resting box trap) were used and their efficacy compared. In addition, large numbers of CDC ovitraps were employed, within 12 months.

**Results:** A total of 4878 adult samples including 22 species covering five genera were collected and identified from traps. It was not revealed any collection of *Ae. albopictus*. Statistical analysis showed no significant difference in meteorological variables between the two periods, the previous report and the current study. There were significant differences in the total number of mosquitoes collected by various traps in the region across different months.

**Conclusion:** The resulting data collected here on the efficiency of the various trap types can be useful for monitoring the densities of mosquito populations, which is an important component of a vector surveillance system. While the presence of *Ae. albopictus* was determined in this potential risk area, there is no evidence for its establishment and further monitoring needs to be carried out.

**Keywords:** *Stegomyia albopicta*; Ovitrap; Sistan and Baluchistan

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## Introduction

Like many countries around the globe, the dengue vector, *Aedes albopictus*, has been recently detected in Iran, with the first report of this species being in 2016 (1). Considering a previous report about the presence and estab-

lishment of this species in the eastern neighbor of Iran, Pakistan, this was not unexpected (2-4). Despite its short flight length, *Ae. albopictus* is considered an invasive species with a rapid potential expansion. It has been

able to spread and establish in various areas from Southeast Asia to America, Europe, Africa and Australian regions (5-7). Due to climatic changes, global warming, and increased international traveling, arboviral diseases and their vectors have been expanded from endemic areas (8). The first Iranian dengue fever case with travel background to Southeast Asia was reported in 2008 (9). The health system expressed its growing concern after DENV-IgG detection in blood donors in southeast of the country, an Oriental ecozone situated in the transit route between East Asia and other countries (10, 11). This area has also been estimated as potentially harboring *Ae. albopictus* (12). This hypothesis was verified by the detection of *Ae. albopictus*, although only five larvae and seven adults were collected (1). Therefore, it is crucial to ascertain the establishment status of this species based on ecological studies and using various sampling methods (13). Indeed, the efficiency of different mosquito traps needs to be investigated across a region to establish an effective vector surveillance system (14). Using a range of trap types, this study tests whether *Ae. albopictus* is established in southeastern Iran and explores the presence of other diurnally active mosquitoes in this potential risk area.

## Materials and Methods

### Study area

This study was carried out in Sistan and Baluchestan, the largest province in Iran (181, 785km<sup>2</sup>). It includes 19 cities, 37 towns, and 9716 villages. It has a long shared border with Pakistan and Afghanistan in the east and the Arabian Sea in the south. Aridity, dust storms, and especially the so-called ‘Wind of 120 Days’ constitute its general climate. Nevertheless, periodic monsoon systems contribute to a diverse climate in the region, especially in southern coastal areas where there are summer rainfalls (11). The maximum average rainfall within 5 years (2011–2015), has been 145.6±89.1mm in the south and 87.9±45.5mm

in the northern part of this province. While the region does not possess numerous water bodies (12), cement water tanks (ponds,) as usual water sources especially in rural areas, could be considered mosquitoes breeding places. The humidity in this region is mainly due to the Bengal Gulf streams, which come to Iran only in the summer (15). Despite the growing of date palm gardens, small farms, and even paddy fields in this province (11), the normalized difference vegetation index (NDVI) shows a thin vegetation cover (12). Based on biogeographical considerations, this province has a unique climate including the Palaearctic and Indo-Malaya Realms (11). This part of the country still struggles with malaria and other vector-borne diseases, resulting in the establishment of a substantial vector surveillance system in the area (16, 17) (Fig. 1).

### Traps description

The CO<sub>2</sub>-baited bednet tarp prepared for this study was 2×2×1.2m in size, with 156 threads per inch mesh size and constructed from polystyrene fabrics. CO<sub>2</sub> gas was released using a pressure reducing regulator with about 0.5 l/min applied in pulses (20s on/40s off) (Fig. 2a). The BG-sentinel 2 trap consisted of a black collapsible fabric container with 40cm high and 36cm in diameter. It has a white gauze lid with a black catch pipe that is opened in case of air suction by an electrical fan. The created airflow, released a BG lure (18) (Fig. 2b). Figure 2c shows the Malaise trap used (height 190–110cm, length 165cm, width 115cm) with a pyramid of white net on a black framework, fabricated from polystyrene. Two sachets of the new BG-sweet scent were used as a mosquito attractant in this trap. The sachets were hung on the inner side of the black framework. Two types of resting box trap (35×35×35cm) were used, depending on the color of the outer walls (black or black-white banded). The internal walls were covered with black cotton fabric. On the front side, there was a 35×15cm entry near the top, while the rest of the front was covered by a

black wooden\cotton wall. A sugar-fermenting yeast solution was applied to attract mosquitoes. The solution was made up of a mixture of dry yeast (7g), sugar (100g) and water (1L) (19) in 1.5L bottles\jars placed in traps or out of them with a connector plastic tube (Fig. 2d). Mosquitoes in this trap (as well as the bednet and Malaise traps) were collected with an oral tube aspirator. The CDC ovitrap is a standardized device for mosquito egg-laying. It involves a black glass jar which is 12.7cm high and 7.6cm in diameter at the top (Fig. 2e). As an oviposition substrate, a wooden paddle (2.7cm long and 1.9cm wide) was placed in the jar, which was filled with water (20). In our study, the glass jar was replaced by a black plastic pot (19×14cm) due to its lower weight and breaking probability.

### Field sampling procedure and statistical comparison

This descriptive–analytic study was conducted from July 2016 to June 2017. In total, five urban (Zahedan, Rask, Nikshahr, Chababhar and Konarak) 12 rural, and three suburban areas were explored for mosquitoes. In our study, suburb means peri-urban which includes outer residential areas of a city. The sampling points were chosen based on a previous study which had resulted in the *Ae. albopictus* collection. The larvae of this species had been detected in Rask (26.28471 °N, 61.40040 °E; elevation 421 meter above sea level [MASL]) and adult samples were collected from Paroomi (25.44267°N, 60.90731°E; elevation 44 MASL) and Vashname-dori (25.45919°N, 60.83179°E; elevation 9 MASL) (1). Besides, the sampling points were exploited from a modeling study conducted in this area covering various subclimates and topographies. Adult sampling was undertaken at 10 points; three were fixed (Rask, Paroomi, Vashname-dori) and seven were varied within an area. The first fixed sampling point was urban, and the other two points were classified as rural areas. Sampling started before noon until half an hour after

dusk. Adult mosquito traps were installed 20–50m apart. During adult sampling, meteorological variables including temperature, humidity and wind speed were recorded every two hours (Fig. 3b). The CO<sub>2</sub>-baited bednet, Malaise and Resting box tarps were checked every 15 minutes for five minutes. However, it was done once only at the end of the sampling day for the BG-sentinel 2 trap. Due to air suction by the electrical fan, the collected mosquitoes could not escape. Like other traps, the BG-sentinel 2 trap is installed outdoors. Trap placement was conducted according to the instruction manual provided by Biogents AG. It was protected from wind, rainfall, and direct sunlight, yet visible to mosquitoes. Besides, the trap was positioned close to the mosquito breeding sites. The collected adult specimens were identified based on an Iranian mosquito key (21). In addition, 505 ovitraps were installed at 31 points (Fig. 3a), including cities, villages, customs areas, and seaports. The paddles were checked every two weeks and suspected cases were moved to the insectary for laboratory assessments. Finally, the obtained data, collected specimens, month, average meteorological variables and trap types were analyzed statistically. Contingency analysis was used to assess the co-occurrence of a species at a location/time point and computed phi from contingency tables as a measure of the strength of species' association of species between different trap types. To this end, generalized linear models (GLMs), were ran, and the data were analyzed as a dichotomous variable in IBM SPSS Statistics 24.

## Results

### Adult sampling results

A total of 4878 adults were collected from various traps, in which 22 species of five genera (including three of the genus *Aedes*, nine *Culex*, eight *Anopheles*, one *Culiseta*, and one *Uranotaenia*) were identified. Overall, *Ae. caballus* (35.5%) and *Culex quinquefasciatus* (23.82%) were the dominant species, followed

by *Cx. sitiens* (17.6%), *Cx. tritaeniorhynchus* (10.9%) and *Ae. caspius* (4.2%) (Table 1). When pooled across species, a clear mosquito collection peak was detected in February, which coincided with a period of low wind speed, low temperature, and high humidity (Fig. 4). Of the 11 meteorological stations scattered throughout the study area, three points were selected. Based on the previous report, *Ae. albopictus* was collected from three sampling sites. The adults had been trapped in Chabahr County in 2013, and the larvae were collected in Nikshahr and Rask Counties in 2009. Thus, meteorological data including the mean temperature as well as mean relative humidity along with precipitation related to these three counties were obtained from the data center of the provincial meteorological organization. Figure 5 shows the monthly comparison of these variables. In December 2013, the maximum rainfall (38.5mm) was recorded in Chabahr station, followed by February of the same year (20.4mm). In 2017, the maximum rainfall occurred in February (68.9mm). The Mann-Whitney test revealed no significant difference concerning these three variables between two years (12 months); 2013 and 2016–2017 ( $P > 0.05$ ), in three larvae and adult sampling sites (Chabahr, Nikshahr, and Rask). Most mosquitoes were collected by CO<sub>2</sub>-baited bednet traps (65.13%), and with a substantial percentage were caught by Malaise traps (34.09%). The Resting-box traps and BG traps only collected 0.51% and 0.27% of the mosquitoes respectively. The percentage of species collected by Malaise and bednet traps tended to be highly correlated (Table 1), whereas it varied in the case of other adult trap types (for instance, BG traps captured a high percentage of *Cx. sitiens*). Contingency tables (Table 2) indicated that the two trap types placed at the same location tended to collect the same species. This was also reflected in the high phi scores associated with these comparisons. When pooled across trap types, the presence/absence of species was only associated with *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* ( $X^2 = 15.90$ ,

$df = 1$ ,  $P < 0.001$ ,  $\phi = 0.375$ ) as well as *Cx. sitiens* and *Ae. caspius* ( $X^2 = 36.04$ ,  $df = 1$ ,  $P < 0.001$ ,  $\phi = 0.565$ ). The data for these four most common species were statistically analyzed. Another common species, *Ae. caballus*, was only detected in rural areas in one period and it was not analyzed further. *Aedes caballus* was only collected in February by both Malaise and bednet trap types. The other species were collected throughout the year (Fig. 6). Bednet and Malaise trap data were combined to evaluate the effects of the three regions (urban, suburban, and rural) along with other environmental variables on the occurrence of the four common species. For *Ae. caspius*, GLM indicated the significant effect of region (Wald  $X^2 = 9.846$ ,  $df = 2$ ,  $P = 0.007$ ), such that this mosquito was more common in urban areas (Fig. 7). Moreover, wind was found to exert a marginally non-significant effect (Wald  $X^2 = 3.458$ ,  $df = 1$ ,  $P = 0.063$ ), such that lower wind speed was associated with a higher probability of positive traps ( $B = 1.221$ , 95% CI 0.066, 2.509). For *Cx. quinquefasciatus*, GLM only confirmed the significant impact of wind speed (Wald  $X^2 = 5.153$ ,  $df = 1$ ,  $P = 0.023$ ), such that lower wind speed again was related to positive traps ( $B = 1.699$ , 95%, CI 0.232, 3.167). Regarding *Cx. tritaeniorhynchus*, the results of GLM exhibited the significant effect of location, as reflected by the impact of latitude (Wald  $X^2 = 6.708$ ,  $df = 1$ ,  $P = 0.010$ ) and longitude (Wald  $X^2 = 6.773$ ,  $df = 1$ ,  $P = 0.009$ ). Additionally, this species was significantly affected by wind speed (Wald  $X^2 = 5.402$ ,  $df = 1$ ,  $P = 0.020$ ). Finally, none of the parameters had a significant role in the occurrence of *Cx. sitiens*.

### Ovitrap results

No *Aedes* eggs and larvae were detected on ovitraps' paddles and waters respectively. Two species, *Cx. tritaeniorhynchus* ( $n = 11$ ) and *Anopheles stephensi* ( $n = 4$ ), were collected from water of ovitraps at two points located in Sarbaz County (26.14118 °N, 61.45337

°E, elevation 329 MASL) and (26.15303°N, 61.44254 °E, elevation 360 MASL).

**Table 1.** The collected mosquito species by trap types, southeastern Iran, Sistan and Baluchestan Province, 2016–2017

Species	Co2-baited Bednet		Malaise		BG		Resting Box		Total	
	n	%	n	%	n	%	n	%	n	%
<i>Aedes caspius</i>	94	3.0	107	6.4	3	12	1	7.7	205	4.20
<i>Ae. caballus</i>	1031	32.5	698	42.0	4	16	1	7.7	1734	35.55
<i>Ae. vexans</i>	2	0.1	1	0.1	0	0	0	0	3	0.06
<i>Culex quinquefasciatus</i>	798	25.1	354	21.3	5	20	5	38.5	1162	23.82
<i>Cx. tritaeniorhynchus</i>	335	10.5	191	11.5	3	12	2	15.4	531	10.89
<i>Cx. pipiens</i>	87	2.7	19	1.1	0	0	3	23	109	2.23
<i>Cx. sitiens</i>	602	18.8	247	14.8	10	40	0	0	859	17.61
<i>Cx. prexigus</i>	38	1.2	10	0.6	0	0	0	0	48	0.98
<i>Cx. bitaniorhynchus</i>	5	0.2	0	0.0	0	0	0	0	5	0.10
<i>Cx. theileri</i>	5	0.2	1	0.1	0	0	0	0	6	0.12
<i>Cx. pseudovishnui</i>	15	0.5	2	0.1	0	0	1	7.7	18	0.37
<i>Cx. siniticus</i>	41	1.3	21	1.3	0	0	0	0	62	1.27
<i>Anopheles stephensi</i>	61	1.9	9	0.5	0	0	0	0	70	1.44
<i>An. culicifacies</i> s.l.	16	0.5	0	0.0	0	0	0	0	16	0.33
<i>An. superpictus</i> s.l.	10	0.3	0	0.0	0	0	0	0	10	0.21
<i>An. turkhudi</i>	1	0.0	0	0.0	0	0	0	0	1	0.02
<i>An. subpictus</i> s.l.	5	0.2	0	0.0	0	0	0	0	5	0.10
<i>An. fluviatilis</i> s.l.	2	0.1	0	0.0	0	0	0	0	2	0.04
<i>An. dthali</i>	1	0.0	0	0.0	0	0	0	0	1	0.02
<i>An. moghulensis</i>	1	0.0	0	0.0	0	0	0	0	1	0.02
<i>Urantaenia unguiculata</i>	2	0.1	0	0.0	0	0	0	0	2	0.04
<i>Culisets longiareolata</i>	25	0.8	3	0.2	0	0	0	0	28	0.57
<b>Total</b>	<b>3177</b>	<b>100</b>	<b>1663</b>	<b>100</b>	<b>25</b>	<b>100</b>	<b>13</b>	<b>100</b>	<b>4878</b>	<b>100</b>



**Fig. 1.** Location of the study area, Sistan and Baluchestan Province, southeastern Iran, surveyed during 2016–2017

**Table 2.** Co-occurrence of mosquitoes from the same species in the two trap types, Sistan and Baluchestan Province, 2016–2017

Species	Present in both B and M*	Absent in both B and M	Only in B	Only in M	X <sup>2</sup> (df= 1), P	Phi
<i>Aedes caspius</i>	26	83	2	2	92.56, <0.001	0.905
<i>Culex quinquefasciatus</i>	58	19	35	1	21.71, <0.001	0.438
<i>Cx. tritaeniorhynchus</i>	29	60	21	3	30.92, <0.001	0.587
<i>Cx. sitiens</i>	17	91	5	0	82.77, <0.001	0.856

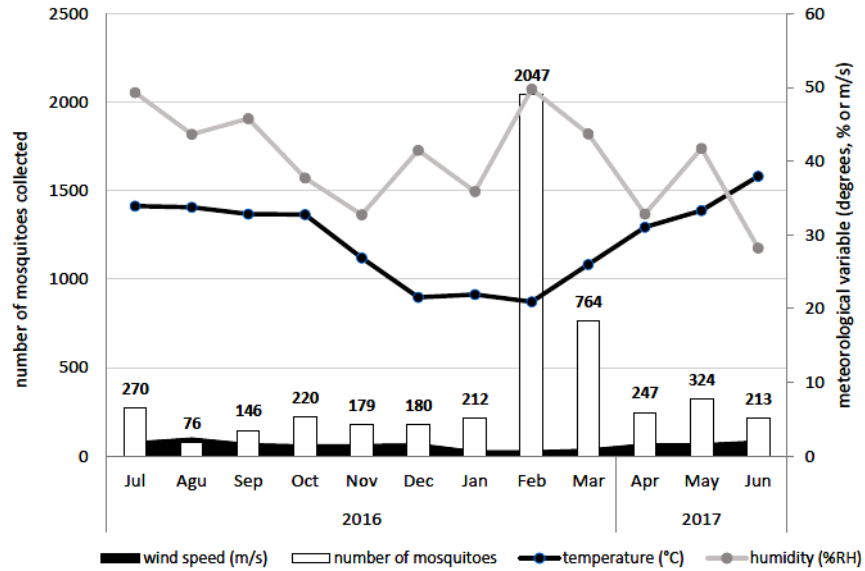
\*B: CO<sub>2</sub>-baited bednet Trap, M: Malaise Trap



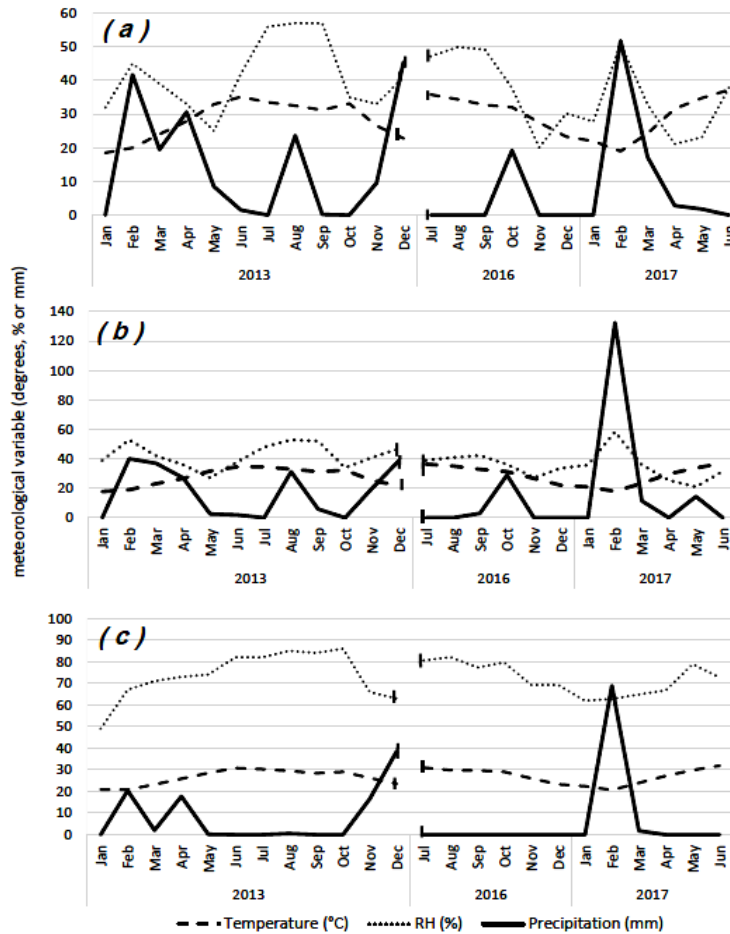
**Fig. 2.** Traps used for mosquito collection, CO<sub>2</sub>-baited bednet tarp (a), Malaise trap (b), 450 resting box (c), BG (d), ovitrap (e), southeastern Iran, Sistan and Baluchestan Province, 2016–2017



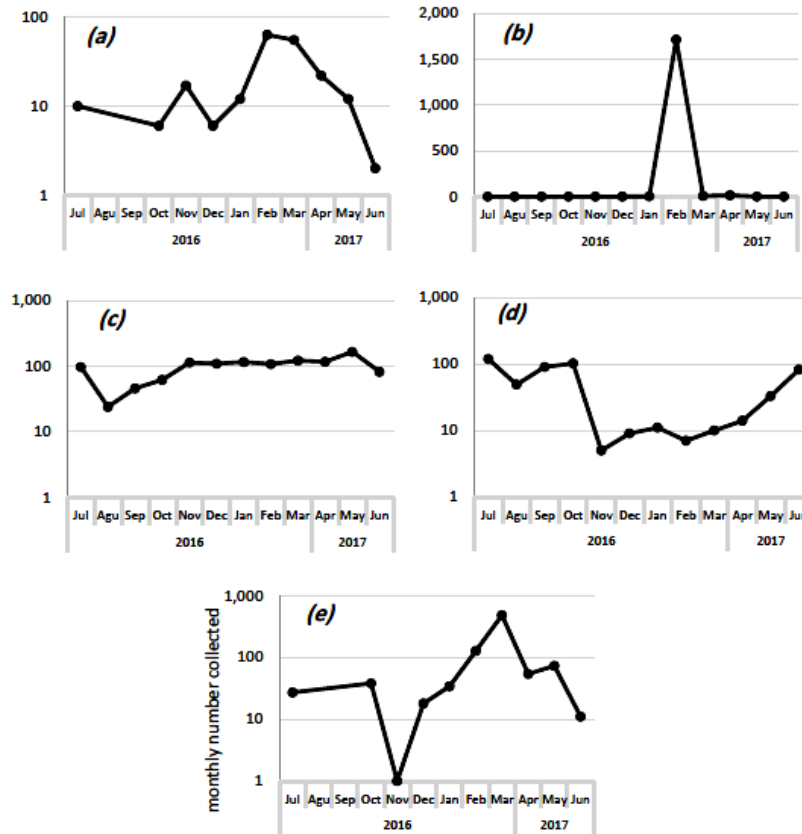
**Fig. 3.** Mosquito sampling sites; Adults (a), Ovitrap (b), southeastern Iran, Sistan and Baluchestan Province, 2016–2017



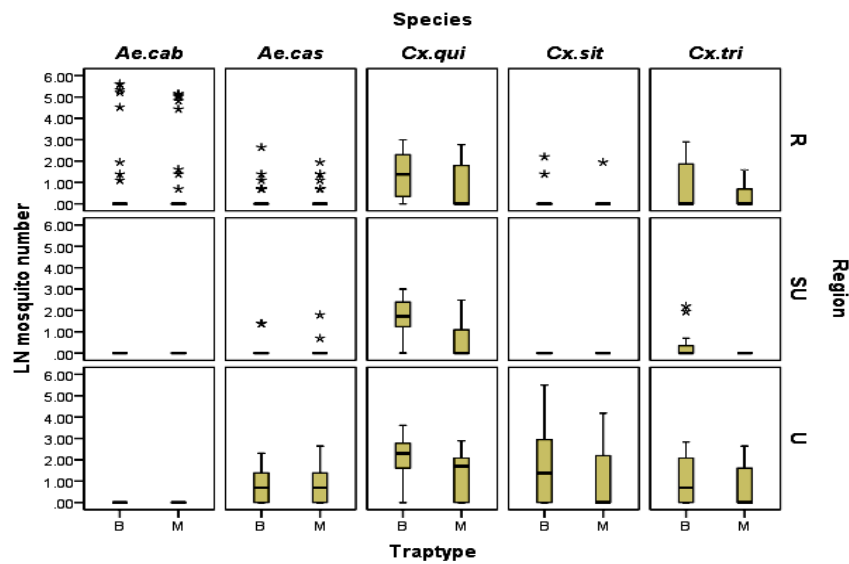
**Fig. 4.** The collected mosquitoes and meteorological variables by month, southeastern Iran, Sistan and Baluchestan Province, 2016–2017



**Fig. 5.** Monthly comparison of the meteorological variables between two years, 2013 and July 2016–June 2017 in three sampling sites: (a) Rask, (b) Nikshahr, (c) Chabahar, southeastern Iran, Sistan and Baluchestan Province, 2016–2017



**Fig. 6.** Monthly number of mosquitoes from traps as an estimate of population density for (a) *Aedes caspius*, (b) *Ae. caballus*, (c) *Culex quinquefasciatus*, (d) *Cx. tritaeniorhynchus* and (e) *Cx. sitiens*. Note log scale apart from (b), Sistan and Baluchestan Province, 2016–2017



**Fig. 7.** Box plots for ln density of five common species by two traps in different areas. (*Ae. cab*) *Aedes caballus*, (*Ae. cas*) *Ae. caspius*, (*Cx. qui*) *Culex quinquefasciatus*, (*Cx. sit*) *Cx. sitiens*, (*Cx. tri*) *Cx. tritaeniorhynchus* are plotted. Ln counts from (R) rural, (SU) suburb, and (U) urban regions are plotted separately. Traps were (B) bednet or (M) Malaise. Asterisks indicate outliers, Sistan and Baluchestan Province, 2016–2017



## Discussion

*Aedes albopictus* is a successful container breeder. Once introduced into an area, its establishment can be considered as evidence. Besides, most control efforts focus on the eradication of this species in case their first detection attempts prove unsuccessful. Meanwhile, this is dependent on the species' environmental adaptability and the quality of control measures applied (22). Our study was conducted in a broad area with imported dengue cases and historical vector collection. Using different mosquitoes sampling methods within 12 months, did not obtain any collection of *Ae. albopictus*. In a similar investigation performed in southern California, surveillance of this species after about one year from discovery indicated no presence of adults or signs of oviposition. In that research, two types of trap were used: battery-operated CDC/CO<sub>2</sub>-baited light traps and ovitraps. It was carried out after a report supporting the collection and detection of significant numbers of *Ae. albopictus* in the cargo containers of lucky bamboo shipped from China. The rapid control response to the first report of this species and the cooperative efforts aimed at eliminating it were regarded as reasons for this success (22). Meanwhile, we do not have any evidence on the eradication efforts carried out by the Iranian health system and their aftereffects on the *Ae. albopictus* community consequently. Nevertheless, some modeling studies have suggested that northern parts of Iran can be susceptible to the establishment of this vector. In a high-resolution map, it was found that enhanced vegetation index (EVI) annual mean, EVI range, annual monthly maximum precipitation, annual monthly minimum precipitation, temperature suitability, and urban as well as peri-urban areas are environmental covariates affecting the occurrence probability of *Ae. albopictus*. Specifically, in order of significance, temperature suitability, minimum precipitation, and EVI demonstrated the most relative contribu-

tions to predicting this map (23). Studies conducted in southeast of Iran have indicated that low vegetation index, high temperature, along with low rainfall associated with this part of the country affect the establishment of *Ae. albopictus* in this region (12). In a previous study in this area, *Ae. albopictus* adults were collected during November/December 2013 after a heavy rainfall (1). Although this species was not collected in our study, the highest peak of mosquito activity was preceded by a torrential rainfall. Some researchers attribute an important role to rainfall in establishing *Ae. albopictus*, such that at least 500 mm of annual rainfall is needed for this species to develop (24, 25). Meanwhile, some studies suggested 290mm of annual rainfall for this to happen (26, 27). Evidently, *Ae. albopictus* could be established in areas with 200–500 mm annual rainfall (5). Despite the correlation between rainfall and the presence of *Ae. albopictus*, some researchers have highlighted the behavior of *Ae. albopictus* as a container mosquito which could exist in the rainfall independently from its breeding sites (28). In a study in India, this species was collected from arid and semi-arid areas. Analogous to the region investigated in the present study, these were plain sandy places with thin vegetation and water stored in containers including a large number of cement and plastic tanks (29). As a result, the risk of *Ae. albopictus* establishment in this area can never be ignored. Although *Ae. albopictus* was not collected in the current study, the efficacy of four types of trap on catching six *Aedes* and *Culex* species were evaluated. Some species such as *Ae. vexans*, *Ae. caballus*, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, and *Cx. sitiens* can be regarded as West Nile fever and Chikungunya vectors (30–34) that occur in Iran (8, 35). In fact, *Cx. quinquefasciatus* and *Ae. vexans* have been previously reported as dominant species in south-east of Iran (36, 37).

Our results showed that the meteorological factors of wind speed and temperature have a direct significant correlation with the total number of collected mosquitoes. In particular, in southern Norway, mosquito abundance displayed a negative association with wind speed, and no mosquitoes were collected at speeds above 7.5m/s (38). Our results confirmed that wind speed affects the collection likelihood of several mosquito species. Monitoring densities of mosquito populations can be performed by various sampling methods to evaluate the vectors' behavior, especially the effects of vector control interventions (39). In the current study, CO<sub>2</sub>-baited bednet trap was associated with the majority of collected mosquitoes. We selected this trap based on a previous study in Iran where it was proved effective in collecting *Ae. albopictus* (1). Also, this trap has been used for sampling in other vector-borne diseases such as dirofilariasis (40) and malaria (41) in Iran. However, no report has been made so far concerning the efficacy of this trap type for mosquito sampling. Compared to other traps, CO<sub>2</sub>-baited bednet trap covers a larger space, possibly increasing its ability to collect mosquitoes especially in a semi-desert climate with low mosquito density. Furthermore, CO<sub>2</sub> used in this trap might have served as an efficient mosquito attractant (42). Most studies on trap efficacy have focused on evaluating BG traps versus other traps. For instance, a study in Germany observed that the BG trap was better at collecting a range of mosquito species than three other types of traps: Heavy Duty Encephalitis Vector Survey trap (EVS trap), Centers for Disease Control miniature light trap (CDC trap) and Mosquito Magnet Patriot Mosquito trap (MM trap). Also contrary to our findings, the widest range of mosquito species was collected by BG traps (43). The field efficacy of the BG trap for collecting of *Ae. albopictus* has been also studied in New Jersey, US, where it collected more mosquitoes compared with the CDC trap and the gravid trap (GT). Therefore, this trap type has been recommended as

an important part of vector monitoring and surveillance programs (44). Although most entomological studies with BG traps have been undertaken in Europe, North and South America, Australia, and Southeast Asia, covering tropical regions or areas with dense vegetation, the poor performance of BG traps in the current study was surprising (44-46). Perhaps this type of trap, especially in the absence of CO<sub>2</sub>, is less effective in a dry climate with a low mosquito density. On the other hand, Malaise traps (47) and bednet traps performed well in our study. There are few published papers on the usage of Malaise traps for mosquitoes in tropical areas of the world (48). In the US, Townes-type Malaise traps (49) were used for about five months to collect mosquitoes, with *Aedes* species being the most widely collected, followed by *Culex* species. Vision as well as dark color can play an important role in the attraction of some insects such as Tabanid flies and mosquitoes (50). Mosquitoes possibly showed a positive response to the Malaise trap due to its black color and its BG lure as an attractant. Since this trap and the bednet traps tend to catch common mosquitoes in similar proportions at the same locations and times, both trap types could be useful for general mosquito surveys. The so-called resting Bbox trap has been applied and modified for sampling malaria vectors, particularly outdoor-resting mosquito species (39). We modified it to monitor *Ae. albopictus* with a strong exophilic (resting) behavior (51). However, in our study, this trap did not perform well compared to others. This trap is marked by a low efficiency in collecting *Cs. inornata* (48). There are few published papers regarding the usage of resting box traps for *Aedes* sampling. In Thailand, two types of trap consisting of short boxes (45cm) and tall boxes (90cm) were placed inside houses (52). The short boxes, whose size was close to the one employed in our trap, attracted relatively more females. Perhaps this type of trap is an efficient method for sampling *Ae. aegypti* rather than the species collected here. Ovitrap

are commonly used for sampling container-breeding mosquitoes such as *Ae. albopictus* and *Ae. aegypti* (53), even though their efficacy in catching malaria vectors has also been evaluated (54). In the current study, *Ae. albopictus* larvae were not detected in ovitraps, but the two species of *Cx. tritaeniorhynchus* and *An. stephensi* were occasionally observed. In studies designed for mosquito control, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, and *An. stephensi* have been reported alone or along with *Ae. aegypti* in ovitraps (53, 55–58). Ovitrap could, therefore, be used in surveillance but their low return rate needs to be taken into account, given that > 500 ovitraps were placed outdoors in this study.

## Conclusion

Data collected here on the efficiency of various trap types can be useful for monitoring densities of mosquito populations, which is an important component of a vector surveillance system. Although *Ae. albopictus* had been previously collected from the study area, its establishment is now questionable given that we could not collect it despite substantial effort. Further inquiry into *Ae. albopictus* establishment in this potential risk area should be rewarding. Eventually, in case of local transmission of dengue, studies on the potential transmission of other *Aedes* species should be considered in any new situation.

## Acknowledgements

This study was financially supported by Tehran University of Medical Sciences with the Grant Number 9121260011 and partially by Ministry of Health and Medical Education under code number of NIMAD 982984. The study was also supported by an NHMRC Fellowship to AAH. This research could not be accomplished without the assistance of our colleagues in Zahedan and Iranshahr health deputy. Hence, we would like to express our deep-

est appreciation to all those who provided us with the possibility to do this study. We are also grateful to Abdolghafar Hasanzehi and Abdolmohsen Parvin, the health staff in charge of CDC dengue management in Zahedan and Iranshahr Universities of Medical Sciences. We thank Farooq Askani, Aghil Birnoor, Rasulbakhsh Raeisi, Ebrahim Badroozeh, Salim Nabatzehi, Shahab Dehghani, and Eng. Noormohammad Nabatzehi for their assistance in field sampling. In addition, thanks go to Dr Asghar Talbalaghi, freelance entomologist and consultant in IPM in Italy, for his invaluable comments during sampling. Last but not least, we thank Eng. Hamid Reza Behjati in Sistan and Baluchestan Meteorological Organization for providing weather data.

The authors declare that there is no conflict of interest.

## References

1. Doosti S, Yaghoobi-Ershadi MR, Schaffner F, Moosa-Kazemi SH, Akbarzadeh K, Gooya MM, Vatandoost H, Shirzadi MR, Mosta-Favi E (2016) Mosquito surveillance and the first record of the invasive mosquito species *Aedes* (*Stegomyia*) *albopictus* (Skuse) (Diptera: Culicidae) in Southern Iran. *Iran J Public Health*. 45(8): 1064–1073
2. Khan J, Munir W, Khan B, Ahmad Z, Shams W, Khan A (2015) Dengue outbreak 2013: Clinical profile of patients presenting at DHQ Burner and THQ Shangla, Khyber Pakhtunkhwa, Pakistan. *Immu Dis*. 3: 1–4.
3. Mukhtar M, Tahir Z, Baloch TM, Mansoor F, Kamran J (2011) Entomological investigations of dengue vectors in epidemic-prone districts of Pakistan during 2006–2010. *Dengue Bull*. 35: 99–115.
4. Suleman M, Faryal R, Aamir UB, Alam MM, Nisar N, Sharif S, Shaukat S, Khurshid A, Angez M, Umair M (2016) Dengue

- outbreak in Swat and Mansehra, Pakistan 2013: an epidemiological and diagnostic perspective. *Asian Pac J Trop Med.* 9(4): 380–384.
5. Bueno-Marí R, Jiménez-Peydró R (2015) First observations of homodynamic populations of *Aedes albopictus* (Skuse) in Southwest Europe. *J Vector Borne Dis.* 52(2): 175–181.
  6. Roiz D, Neteler M, Castellani C, Arnoldi D, Rizzoli A (2011) Climatic factors driving invasion of the tiger mosquito (*Aedes albopictus*) into new areas of Trentino, northern Italy. *PloS One.* 6(4): e14800.
  7. Talbalaghi A, Moutailler S, Vazeille M, Failloux AB (2010) Are *Aedes albopictus* or other mosquito species from northern Italy competent to sustain new arboviral outbreaks?. *Med Vet Entomol.* 24(1): 83–87.
  8. Ardalan M, Chinikar S, Shoja MM (2014) Hemorrhagic Fever with renal syndrome and its history in Iran. *Iran J Kidney Dis.* 8(6): 438–441
  9. Chinikar S, Ghiasi SM, Moradi A, Madihi SR (2010) Laboratory detection facility of dengue fever (df) in iran: the first imported case. *Int J Infect Dis.* 8(1): 1–2.
  10. Aghaie A, Aaskov J, Chinikar S, Niedrig M, Banazadeh S, Mohammadpour HK (2014) Frequency of dengue virus infection in blood donors in Sistan and Baluchestan Province in Iran. *Transfus Apher Sci.* 50(1): 59–62.
  11. Nejadi J, Saghafipour A, Vatandoost H, Moosa-Kazemi SH, Motevalli Haghi A, Sanei-Dehkordi A (2018) Bionomics of *Anopheles subpictus* (Diptera: Culicidae) in a malaria endemic area, southeastern Iran. *J Med Entomol.* 55 (5): 1182–1187.
  12. Nejadi J, Bueno-Marí R, Collantes F, Hanafi-Bojd AA, Vatandoost H, Charrahy Z, Tabatabaei SM, Yaghoobi-Ershadi MR, Hasanzehi A, Shirzadi MR (2017) Potential risk areas of *Aedes albopictus* in south-eastern Iran: a vector of dengue Fever, Zika and chikungunya. *Front Microbiol.* 8: 1–12.
  13. Urbanelli S, Bellini R, Carrieri M, Sallicandro P, Celli G (2000) Population structure of *Aedes albopictus* (Skuse): the mosquito which is colonizing Mediterranean countries. *Heredity.* 84(3): 331–337.
  14. Pezzin A, Sy V, Puggioli A, Veronesi R, Carrieri M, Maccagnani B, Bellini R (2016) Comparative study on the effectiveness of different mosquito traps in arbovirus surveillance with a focus on WNV detection. *Acta Trop.* 153: 93–100.
  15. Nejadi J, Tabatabaei SM, Salehi M, Saghafipour A, Mozafari E (2017) Some probable factors affecting the malaria situation before and at the beginning of a pre-elimination program in south-eastern Iran. *J Parasit Dis.* 41(2): 503–509.
  16. Vatandoost H, Emami S, Oshaghi M, Abai M, Raeisi A, Piazzak N, Mahmoodi M, Akbarzadeh K, Sartipi M (2011) Ecology of malaria vector *Anopheles culicifacies* in a malarious area of Sistan va Baluchestan Province, south-east Islamic Republic of Iran. *East Mediterr Health J.* 17(5): 439–445.
  17. Hemami MR, Sari AA, Raeisi A, Vatandoost H, Majdzadeh R (2013) Malaria elimination in Iran, importance and challenges. *Int J Prev Med.* 4(1): 894.
  18. Biogents (2017) Mosquito Trap BG-Sentinel 2 Co2 Instruction Manual. Regensburg, Germany.
  19. Smallegange RC, Schmied WH, van Roey KJ, Verhulst NO, Spitzen J, Mukabana WR, Takken W (2010) Sugar-fermenting yeast as an organic source of carbon dioxide to attract the malaria

- mosquito *Anopheles gambiae*. Malar J. 9(1): 292.
20. Reiter P, Colon M (1991) Enhancement of the CDC ovitrap with hay infusions for daily monitoring of *Aedes aegypti* populations. J Am Mosq Control Assoc. 7(1): 52–55.
  21. Azari-Hamidian S, Harbach RE (2009) Keys to the adult females and fourth-instar larvae of the mosquitoes of Iran (Diptera: Culicidae). Zootaxa. 2078(1): 1–33.
  22. Madon MB, Hazelrigg JE, Shaw MW, Klueh S, Mulla MS (2003) Has *Aedes albopictus* established in California?. J Am Mosq Control Assoc. 19(4): 297–300.
  23. Kraemer MU, Sinka ME, Duda KA, Mylne AQ, Shearer FM, Barker CM, Moore CG, Carvalho RG, Coelho GE, Van Bortel W (2015) The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. Elife. 4: e08347.
  24. Medlock JM, Avenell D, Barrass I, Leach S (2006) Analysis of the potential for survival and seasonal activity of *Aedes albopictus* (Diptera: Culicidae) in the United Kingdom. J Vector Ecol. 31(2): 292–304.
  25. Mitchell C (1995) Geographic spread of *Aedes albopictus* and potential for involvement in arbovirus cycles in the Mediterranean basin. J Vector Ecol. 20(1): 44–58.
  26. Benedict MQ, Levine RS, Hawley WA, Lounibos LP (2007) Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. Vector Borne Zoonotic Dis. 7(1): 76–85.
  27. Severini F, Di ML, Toma L, Romi R (2008) *Aedes albopictus* in Rome: results and perspectives after 10 years of monitoring. Parassitologia. 50(1–2): 121–123.
  28. Waldock J, Chandra NL, Lelieveld J, Proestos Y, Michael E, Christophides G, Parham PE (2013) The role of environmental variables on *Aedes albopictus* biology and chikungunya epidemiology. Pathog Glob Health. 107(5): 224–241.
  29. Sarfraz MS, Tripathi NK, Faruque FS, Bajwa UI, Kitamoto A, Souris M (2014) Mapping urban and peri-urban breeding habitats of *Aedes* mosquitoes using a fuzzy analytical hierarchical process based on climatic and physical parameters. Geospat Health. 8(3): 685–697.
  30. Huang YM, Rueda LM (2014) A pictorial key to the species of *Aedes* (*Ochlerotatus* and *Coetzeomyia*) in the Afrotropical Region (Diptera: Culicidae). Zootaxa. 3754(5): 592–600.
  31. Bernard KA, Maffei JG, Jones SA, Kauffman EB, Ebel G, Dupuis A, Ngo KA, Nicholas DC, Young DM, Shi P-Y (2001) West Nile virus infection in birds and mosquitoes, New York State, 2000. Emerg Infect Dis. 7(4): 679–684.
  32. Chapman H, Kay B, Ritchie S, Van den Hurk A, Hughes J (2000) Definition of species in the *Culex sitiens* subgroup (Diptera: Culicidae) from Papua New Guinea and Australia. J Med Entomol. 37(5): 736–742.
  33. Glaser RL, Meola MA (2010) The native *Wolbachia* endosymbionts of *Drosophila melanogaster* and *Culex quinquefasciatus* increase host resistance to West Nile virus infection. PloS One. 5(8): e11977.
  34. Hubalek Z, Halouzka J (1999) West Nile fever, a reemerging mosquito-borne viral disease in Europe. Emerg Infect Dis. 5(5): 643–650.
  35. Ahmadnejad F, Otarod V, Fallah M, Lowenski S, Sedighi-Moghaddam R, Zavareh A, Durand B, Lecollinet S, Sabatier P (2011) Spread of West Nile virus in Iran: a cross-sectional serosurvey in equines, 2008–2009. Epidemiol Infect. 139(10): 1587–1593.

36. Dehghan H, Sadraei J, Moosa-Kazemi SH (2011) The morphological variations of *Culex pipiens* (Diptera: Culicidae) in central Iran. *Asian Pac J Trop Med.* 4(3): 215–219.
37. Yaghoobi-Ershadi M, Doosti S, Schaffner F, Moosa-Kazemi S, Akbarzadeh K, Yaghoobi-Ershadi N (2017) Morphological studies on adult mosquitoes (Diptera: Culicidae) and first report of the potential Zika virus vector *Aedes (Stegomyia) unilineatus* (Theobald, 1906) in Iran. *Bull Soc Pathol Exot.* 110(2): 116–121.
38. Hagemoen RIM, Reimers E (2002) Reindeer summer activity pattern in relation to weather and insect harassment. *J Anim Ecol.* 71(5): 883–892.
39. Pombi M, Guelbeogo WM, Kreppel K, Calzetta M, Traoré A, Sanou A, Ranson H, Ferguson HM, Sagnon NF, Della Torre A (2014) The Sticky Resting Box, a new tool for studying resting behaviour of Afrotropical malaria vectors. *Parasit Vectors.* 7(1): 247.
40. Azari Hamidian S, MR YE, Javadian E, Abai M, Mobedi I, Linton YM, Harbach R (2009) Distribution and ecology of mosquitoes in a focus of dirofilariasis in northwestern Iran, with the first finding of filarial larvae in naturally infected local mosquitoes. *Med Vet Entomol.* 23(2): 111–121.
41. Moosa-Kazemi SH, Firoozfar F (2016) Bionomic studies of the mosquitoes (Diptera: Culicidae) in Kermanshah Province, Western Iran. *Life Sci J.* 13(10): 50–55.
42. Gillies M (1980) The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a review. *Bull Entomol Res.* 70(4): 525–532.
43. Lühken R, Pfitzner WP, Börstler J, Garms R, Huber K, Schork N, Steinke S, Kiel E, Becker N, Tannich E (2014) Field evaluation of four widely used mosquito traps in central Europe. *Parasit Vectors.* 7(1): 268.
44. Farajollahi A, Kesavaraju B, Price DC, Williams GM, Healy SP, Gaugler R, Nelder MP (2009) Field efficacy of BG-sentinel and industry-standard traps for *Aedes albopictus* (Diptera: Culicidae) and West Nile virus surveillance. *J Med Entomol.* 46(4): 919–925.
45. Ritchie SA, Moore P, Carruthers M, Williams C, Montgomery B, Foley P, Ahboo S, Van Den Hurk AF, Lindsay MD, Cooper B (2006) Discovery of a widespread infestation of *Aedes albopictus* in the Torres Strait, Australia. *J Am Mosq Control Assoc.* 22(3): 358–365.
46. Hoffmann A, Montgomery B, Popovici J, Iturbe-Ormaetxe I, Johnson P, Muzzi F, Greenfield M, Durkan M, Leong Y, Dong Y (2011) Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature.* 476(7361): 454–461.
47. Malaise R (1937) A new insect-trap. *Entomol Tidskr.* 58: 148–160.
48. Service M (1993) *Mosquito Ecology Field Sampling Methods.* Springer, Switzerland.
49. Townes H (1962) Design for a Malaise trap. *Proc Ent Soc Wash.* 64: 253–262.
50. Van Breugel F, Riffell J, Fairhall A, Dickinson MH (2015) Mosquitoes use vision to associate odor plumes with thermal targets. *Curr Biol.* 25(16): 2123–2129.
51. Delatte H, Desvars A, Bouétard A, Bord S, Gimonneau G, Vourc'h G, Fontenille D (2010) Blood-feeding behavior of *Aedes albopictus*, a vector of Chikungunya on La Réunion. *Vector Borne Zoonotic Dis.* 10(3): 249–258.
52. Edman J, Kittayapong P, Linthicum K, Scott T (1997) Attractant resting boxes for rapid collection and surveillance of *Aedes aegypti* (L.) inside houses. *J Am*

- Mosq Control Assoc. 13(1): 24–27.
53. Ritchie SA, Long S, Hart A, Webb CE, Russell RC (2003) An adulticidal sticky ovitrap for sampling container-breeding mosquitoes. *J Am Mosq Control Assoc.* 19(3): 235–242.
54. Elango G, Zahir AA, Bagavan A, Kamaraj C, Rajakumar G, Santhoshkumar T, Marimuthu S, Rahuman AA (2011) Efficacy of indigenous plant extracts on the malaria vector *Anopheles subpictus* Grassi (Diptera: Culicidae). *Indian J Med Res.* 134(3): 375–379.
55. Surendran SN, Jude PJ, Thavaranjit AC, Eswaramohan T, Vinobaba M, Ramasamy R (2013) Predatory efficacy of *Culex (Lutzia) fuscanus* on mosquito vectors of human diseases in Sri Lanka. *J Am Mosq Control Assoc.* 29(2): 168–170.
56. Elizondo-Quiroga A, Flores-Suarez A, Elizondo-Quiroga D, Ponce-Garcia G, Blitvich BJ, Contreras-Cordero JF, Gonzalez-Rojas JJ, Mercado-Hernandez R, Beaty BJ, Fernandez-Salas I (2006) Gonotrophic cycle and survivorship of *Culex quinquefasciatus* (Diptera: Culicidae) using sticky ovitraps in Monterrey, northeastern Mexico. *J Am Mosq Control Assoc.* 22(1): 10–14.
57. Seenivasagan T, Sharma KR, Prakash S (2012) Electroantennogram, flight orientation and oviposition responses of *Anopheles stephensi* and *Aedes aegypti* to a fatty acid ester-propyl octadecanoate. *Acta Trop.* 124(1): 54–61.
58. Hesson JC, Ignell R, Hill SR, Östman Ö, Lundström JO (2015) Trapping biases of *Culex torrentium* and *Culex pipiens* revealed by comparison of captures in CDC traps, ovitraps, and gravid traps. *J Vector Ecol.* 40(1): 158–163.