Article

Evaluating the efficacy of Olyset® nets in the laboratory and trap surveillance of *Aedes* mosquitoes

Danilo A. Gualberto[1], **Cesar G. Demayo**[2]
[1]Biology Department, College of Arts and Sciences, Xavier University-Ateneo de Cagayan, 73 Corrales Avenue, Cagayan de Oro City, 9000 Misamis Oriental, Philippines
[2]Department of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology, 9200 Iligan City Philippines
E-mails: dgualberto@xu.edu.ph, cgdemayo@gmail.com

Received 2 April 2022; *Accepted 10 May 2022*; *Published online 31 May 2022*; *Published 1 September 2022*

Abstract
With Chikungunya and Zika in the Philippines, there is an urgent need for effective methods to control *Aedes aegypti* and *Aedes albopictus* in as much as these mosquitoes are not only vectors of Dengue but also of Chikungunya and Zika. Evaluation of the efficacy of Olyset® nets was done in two approaches: (1) laboratory WHO cone bioassay on Olyset® net and (2) comparative mosquito trap surveillance of dengue vector mosquitoes in DepEd classrooms with and without Olyset® nets. During laboratory cone bioassay, % knockdown and % mortalities were recorded for *Ae. aegypti* and *Ae. albopictus.* Penetrability tests were also done on Olyset® nets. During comparative mosquito trap surveillance, the total *Aedes* capture in classrooms with Olyset® nets was compared to *Aedes* capture in classrooms without Olyset® nets. *Ae. aegypti* showed A 54% percent knockdown and 63% mortality *Ae. albopictus* showed 77% knockdown, and 79% mortality was shown in cone bioassay of Olyset® nets. Penetrability test on Olyset® nets showed 35% *Ae. aegypti* and 34% *Ae. albopictus* crossed the nets, but only 10% in *Ae. aegypti* and 6% in *Ae. albopictus* survived the crossing. Female adult and larval *Aedes* capture differed significantly between classrooms with Olyset® nets and those without Olyset® nets. But the observed lack of screen doors and poor physical integrity of Olyset® nets in classrooms might compromise its efficacy. Based on WHO 98% susceptibility test criterion, there was probable insecticide resistance in *Aedes* mosquitoes to 2% permethrin in Olyset® nets. In the classroom mosquito surveillance, significant differences of *Aedes* capture between classroom groups may have been obtained, but other observations indicated compromised efficacy of the Olyset® nets.

Keywords Olyset® nets; knockdown; penetrability; surveillance.

Arthropods
ISSN 2224-4255
URL: http://www.iaees.org/publications/journals/arthropods/online-version.asp
RSS: http://www.iaees.org/publications/journals/arthropods/rss.xml
E-mail: arthropods@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

With the emergence of Chikungunya and Zika in the Philippines, there is an urgent need for effective methods

Olyset® Net (ITS) in public schools as an intervention against *Aedes* vector mosquitoes. According to DOH-RX, Olyset® nets were first supplied to DepEd elementary schools in 2009 as a pilot program for the first evaluation of the insecticide-treated screens.

2 Materials and Methods

2.1 Establishment of insectary

Adult *Ae. aegypti* and *Ae. albopictus* were required for the cone bioassay of Olyset® in this study; hence adult mosquitoes were continuously reared from wild-caught larvae and pupae collected from tire and bamboo ovitraps installed in an open field. While at their pupal stages, *Ae. aegypti* and *Ae. albopictus* were laid in shallow trays, separated by sex and species, and then transferred to different rearing boxes equipped with screened tops and sleeves for easy extraction of adults. The adult *Aedes* mosquitoes were collected from hatching boxes using a battery-operated vacuum cleaner and separately transferred based on species and sex to 12 in x 12 in cages. The method used a handheld bulb aspirator with clear tubes to confirm the appropriate species based on scutal markings and other morphological traits by Savage' method (Savage and Smith, 1994). The *Aedes* adult mosquitoes were sustained with 10%-sucrose soaked in flat cotton pads that were laid at the cage tops. To ensure a continuous supply of adult mosquitoes, the male and female adults who emerged from wild-caught larvae were bred in cages in a makeshift insectary. Before egg-laying induction of *Aedes* females, these were sugar-deprived for 2-3 days in their separate cages, feeding them only water while maintaining the 10%-sucrose feeding for the males. At late afternoon hours, the female *Aedes* mosquitoes were blood-fed with immobilized chicken and, after an hour, transferred to another cage for breeding with males of the same species. Small and shallow water-filled glassware with filter paper cones were installed in the same enclosure to collect viable eggs. The eggs were then induced in simultaneous hatching in deoxygenated 10% nutrient broth, and the larvae were transferred to rearing bottles. After roughly one-week, cohorts of emerged adult *Aedes* mosquitoes were sorted by sex, and the females were transferred to separate cages to be used for cone bioassay.

2.2 Laboratory cone bioassay of Olyset®

The cone bioassay, a WHO method of insecticide evaluation (WHO, 2013), was used to assess the effectiveness of the Olyset® nets in public elementary classrooms of Cagayan de Oro City. The test, therefore, aimed to evaluate the insecticidal efficacy of Olyset® on *Ae. aegypti* and *Ae. albopictus* strains of mosquitoes in Cagayan de Oro when they came into contact with it compared to the same species exposed to untreated nets. The null hypothesis tested here was that the Olyset® nets had the same physically limiting effect on *Ae. aegypti* and *Ae. albopictus* as ordinary mosquito nets produced.

Initially, the cone bioassay was intended to be done on-site. But after attempts of cone bioassay "on-site," it was surprisingly discovered that the laboratory-reared *Aedes* adult mosquitoes brought "on-site" had uncanny abilities to squeeze through the mesh-holes of the Olyset® nets and enter the classrooms! It was decided from then on that the cone bioassay of the Olyset® nets had to be done in a laboratory setting.

In the laboratory, cone bioassay was performed on 28 cm x 28 cm cuttings of Olyset® that were stapled to 25 cm x 25 cm frames of 2cmx2cm wooden sticks. To include observation of the "penetrant" adult mosquitoes, additional tulle nets with smaller mesh sizes were attached to the front and back of the framed Olyset® nets. Four WHO bioassay cones were fixed to the framed Olyset® by firmly connecting them to the Net using ordinary hairpins. Olyset® nets at 1, 4, and 6 years-old usages were tested with cone bioassay. The framed Olyset® nets were held upright by pairs of 4-inch long ½-in aluminum angle bars as a base. The bioassay setups were preferably held at 90° instead of the recommended 60° angle to simulate the actual orientation of the ITS in the classroom (Owusu et al., 2015). Getting the natural responses of the test animals was more important than maximizing the results.

Using a handheld bulb aspirator, five 3-5 days old, non-blood fed *Ae. aegypti* and *Ae. albopictus* were separately introduced into each of the four WHO cones of the bioassay setup. Exposure of each batch of five *Ae. aegypti* and five *Ae. albopictus* to Olyset® were done separately for 3 minutes, according to the WHO cone bioassay procedure. After 3 minutes, the *Aedes* mosquitoes were removed using a handheld bulb aspirator and transferred to small screen-topped plastic containers with proper labels. "Penetrant" mosquitoes were transferred to separate containers that were also labeled. The mosquitoes were stored and sustained with 10% sucrose for 48-72 hours. After 60 minutes, percentage knockdown and mortality after 24 hours were observed and recorded. Mosquitoes were scored as "alive" if they could fly (irrespective of the number of legs still intact), "dead", or "knocked down", if immobile or incapable of flying or standing in a coordinated manner, and "penetrant" or "non-penetrant, if they crossed or not the Olyset® nets during cone bioassay.

Cone bioassays were conducted on 1, 3, and 6 years old Olyset® net material was obtained from property custodians through the school principal's approval. Untreated nets with identical mesh sizes were used as a control and were tested during each bioassay. The bioassays were performed at room temperature (25.9–29°C), and relative humidity ranges 58–80%. Interpretation of results was based on WHO bioassay guidelines, which consider that a population is (1) fully susceptible if the final percentage mortality is >98% or higher, (2) incipient resistant if 80-97% mortality is observed, and (3) resistant if mortality is <80%.

2.3 *Aedes* mosquito surveillance in Olyset® and non-Olyset® classrooms

After complying with entry protocols through the DepEd authorities, three (3) surveillance adult-larval mosquito traps were installed in each of five randomly selected classrooms that had Olyset® on their windows (ITS classrooms) and another set of 3 mosquito traps in each of five randomly selected classrooms without Olyset® (Non-ITS classroom). A total of seventy surveillance mosquito traps were installed in seven DepEd elementary schools, with a particular preference for kinder and grade one elementary classroom. The null hypothesis of this surveillance was that there was no difference in *Aedes* mosquito abundance between DepEd classrooms with and without Olyset® nets.

A sampling of mosquitoes (adult and larvae) was done twice for two months in each DepEd elementary school sentinel classroom (Fig. 1). After two months, the surveillance was moved on to another selected school for another two months until all seven schools were covered. The surveillance tool used in the ITS- and non-ITS classrooms were the innovative adult-larval traps.

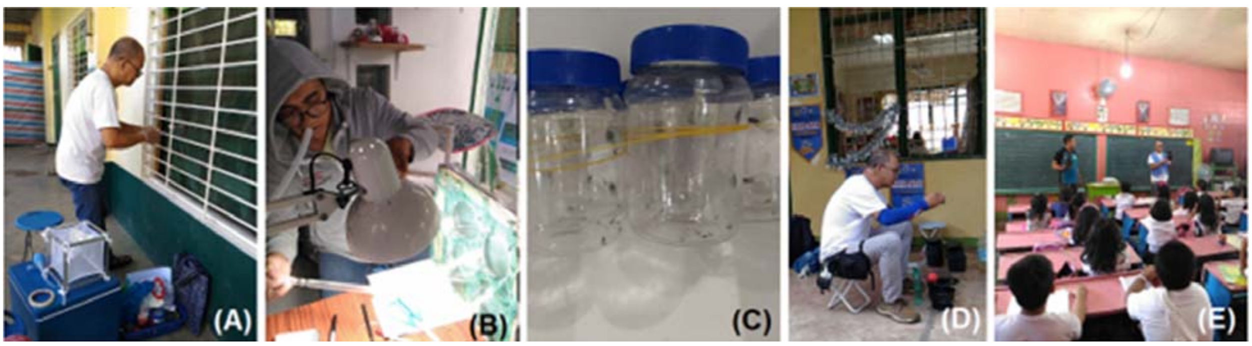

Fig. 1 (A) Field cone bioassay on Olyset® at DepEd school, (B) Laboratory cone bioassay, (C) Mosquito containers after cone bioassay, (D) Mosquito sampling at DepEd school with adult-larval traps, and (E) Student orientation about mosquito trapping in classrooms.

The Adult-Larval Trap (aka ALTrap) was primarily constructed of black, polyethylene plastic dippers with handles sawn-off that was arranged one on top of the other, with their open brims facing each other. One unit of each trap consisted of two parts: (1) an upper adult mosquito-capturing portion and (2) a lower larvae-capturing part. The top portion of the trap contains non-drying glue laid over a stiff board (glue board) inside its walls. Any insect in flight or resting would get stuck to this glue board and would not be able to escape. The lower part of the trap is filled with 1.4 L of 2-3 wks old stored water, the surface of which is covered by a floating ring of sliced foam and screen. Any gravid (egg-laying) female mosquito will be able to deposit its eggs inside its lower walls. Still, when these larvae develop and emerge as an adult, the emerging adult mosquitoes will be drowned under the floating sliced foam and screen. The novelty of this trap is that it can capture both adult mosquitoes and their larvae. It is beneficial for controlling dengue vector mosquitoes inside rooms and outdoors, but it is also helpful for surveillance.

During sampling from the mosquito traps, the glue board containing adhered adult mosquitoes was collected and stored in re-usable plastic cellophane and labeled appropriately. If present at the bottom portion of the adult-larval trap, mosquito larvae were also collected from the mosquito traps, transferred to 4 ml sealable tubes, and labeled appropriately.

In the laboratory, the adhered insects in the glue boards and mosquito larvae were observed, identified, and photo-documented under the Andonstar inspection microscope. Samples from the adult-larval mosquito traps were classified and recorded as 'males' and 'females' of either *Ae. aegypti*, *Ae. albopic*tus, or non-target mosquitoes (mosquitoes not recognized locally and globally as dengue vector mosquitoes). Non-mosquito samples, such as leafhoppers, midges, termites, et cetera, were noted and recorded but were not included in the computation of mosquito abundance.

3 Results

3.1 Overall knockdown and mortality of *Aedes* mosquitoes exposed to Olyset™

A total of 1,200 3-5 days old, unfed adult female *Aedes* mosquitoes were used for WHO cone bioassay on the Olyset® nets. The mosquitoes were segregated into four replicates of 5, each in 5 treatment groups and one control group. A total of 600 adults, female *Ae. aegypti,* and 600 *Ae. albopictus* were sequentially exposed in batches of five individuals to the permethrin-impregnated Olyset® nets for 3 mins cone bioassay. In all treatment groups of *Ae. aegypti*, 52.3% died 24 hours after a 3-minutes exposure to Olyset® nets and a 54% knockdown for the first 60 mins. In *Ae. albopictus*, 81.8% died 24 hours after 3-mins Olyset® exposure and a 75.8% knockdown at the first 60 mins. No Abbott adjustments were necessary for the control groups because mortality was less than 10%.

3.2 Knockdown and mortality of *Aedes* mosquitoes to Olyset™ at various lengths of usage

The average percent knockdown and percent mortality for *Ae. aegypti* and *Ae. albopictus* resulting from cone bioassay of Olyset® nets is given below (Table 1).

Table 1 Percent knockdown and mortality of *Ae. aegypti* and *Ae. albopictus* in cone bioassay of Olyset® nets at different years of usage.

Olyset® Bioassay	*Ae. aegypti*		*Ae. albopictus*	
Years of usage	% Knockdown	% Mortality	% Knockdown	% Mortality
1	52	51	79	81
3	57	76	85	76
6	53	62	67	81
Average	*54*	*63*	*77*	*79*

Two-way ANOVA of the mortality scores between treatments and control groups in *Ae. aegypti* and *Ae. albopictus* showed significant differences between species (average p=0.015<0.05). Two-way ANOVA of means of mortality scores of *Ae. aegypti* bioassayed to Olyset® net materials of different ages detected no significant differences except between 3- and 6-years old net material (p=0.033<0.05). The sampled Olyset® net materials used in the cone bioassay were clean but were never washed. As pointed out by others, the insecticide's availability did not persist over time (Erlanger et al., 2004; Tami et al., 2004; Vythilingam et al., 1996; N'Guessan et al., 2001). The significant difference in mortalities between 3 and 6 years of usage may likely be due to the time-dependent potency of the Olyset® nets. Many interactions were detected in the 2-way ANOVA test of means between species and years of usage. This lends evidence that the observed mortalities are due to the potency of the Olyset® nets and the inherent susceptibilities of the species in question.

3.2.1 Penetrability of Olyset® nets

During this preliminary evaluation of Olyset® Net, it was surprisingly observed that some individuals of *Ae. aegypti* and *Ae. albopictus* were quite capable of crossing the Olyset® nets (Figs. 2 and 3). During cone bioassay, 35% of *Ae. aegypti* and 34% of *Ae. albopictus* crossed the test nets. Out of these penetrant mosquitoes, 71% in *Ae. aegypti* and 83% in *Ae. albopictus* died.

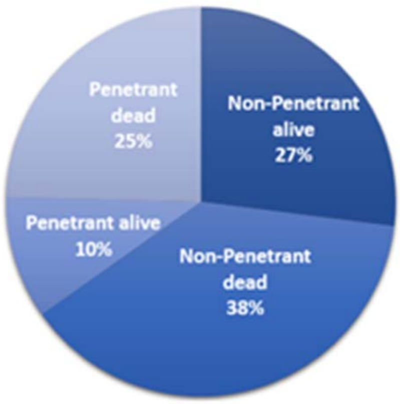

Fig. 2 Penetrant and non-penetrant *Ae. aegypti* during cone bioassay of Olyset® nets.

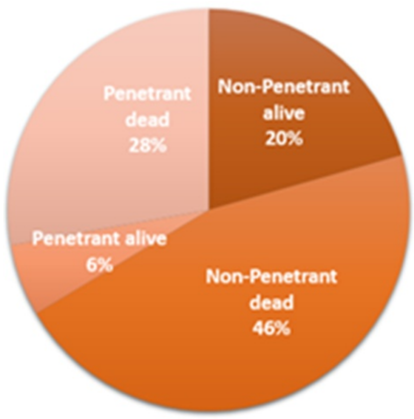

Fig. 3 Penetrant and non-penetrant *Ae. albopictus* during cone bioassay of Olyset® nets.

In a study conducted in Malaysia, the penetrability test on the Olyset® Net yielded 45.2% of *Ae.aegypti* and 19% of *Anopheles maculatus* could cross the 3 mm x 3 mm mesh size of the Olyset® Net (Vythilingam et al., 1996). Mortality in the penetrant *Ae. aegypti* was 25%, and in *Anopheles maculatus,* mortality was 8.9%. In a comparative study on mosquito-penetrability across five different commercial bed nets, penetration success of *Ae. aegypti* was only 13% while *Ae. albopictus* showed 46% crossing Olyset® bednets (Andrade and Cabrini, 2010). Mortality was 100% in all *Ae. aegypti* penetrants, while only 96% of *Ae. albopictus* penetrants died. In a video-aided study in the US, Dickerson et al. (2018) showed that free-flight void entry of *Ae. aegypti* was mainly limited to 10-mm holes because as the hole got smaller (mesh size <10 mm), frequency of access or penetration became impossible.

In the current study, data have shown that out of 1000 *Ae.* mosquitoes exposed to Olyset® nets in cone bioassay, 34.2% (342 individuals) of *Aedes* mosquitoes were able to cross the nets. They crossed the nets not by flying but by crawling erratically and squeezing themselves through the nets. The mesh size of Olyset® nets was only 3-millimeters, but it was not an absolute barrier for the Cagayan de Oro strains of *Ae. aegypti* and *Ae. albopictus*. An adult female *Ae. aegypti* or *Ae. albopictus* exhibited an uncanny way of probing the mesh holes with its proboscis, after which it inserted its head first. Then by pushing and squeezing its thorax across the narrow Olyset® mesh hole, it swiftly jettisons itself across the Net with its fore- and midlegs. In the older (6-years old) softer-fabric version of Olyset® nets, *Aedes* mosquitoes effortlessly crouched through the mesh. During cone bioassay, 5 *Aedes* mosquitoes' test-batch was lost to the other side of the Net in no time!

Following the fate of these Olyset® trespassers in separate holding containers, it was found that only 10% of *Ae. aegypti* and 6% of *Ae. albopictus* survived. Having come into contact with Permethrin throughout most of their body, 25% of *Ae. aegypti* and 28% of *Ae. albopictus* could not last long minutes after the permethrin exposure. The surviving Olyset® net trespassers could have theoretically acted as vectors. It would have been interesting to follow the biting frequency of these surviving *Aedes* mosquitoes were they not needed to be terminated. *Aedes* mosquitoes have uncannily evolved a behavioral strategy that enables them to reach their blood source despite physical and chemical barriers. And based on the concept paper of Sumitomo Chemical Company itself, their design of 4 mm mesh size of the Olyset® Net exploits this trespassing behavior of mosquitoes to maximize exposure to the insecticide released at the surfaces of the net fibers (Okuno and Ito, 2006).

The ratio of Olyset® net percent penetrations by *Ae. aegypti* and *Ae. albopictus* were indistinguishable, as shown in figures 3 and 4, attesting that the populations had equal abilities to cross the nets ($p=0.7659>0.05$). But as scores of survival to permethrin exposure by the penetrants were analyzed, results showed that *Ae. aegypti* survived more than *Ae. albopictus* did ($p=0.04<0.05$). This finding consistently shows the generally low mortality that *Ae. aegypti* exhibited in cone bioassay. Reductions in biting rate had been observed by other researchers in *Anopheles gambiae*, *Anopheles funestus,* and *Culex quinquefasciatus* (N'Guessan et al., 2001)

3.2.2 Surveillance results of mosquito trapping at selected classrooms with and without Olyset®.

A total of 210 adult-larval mosquito traps were installed in sentinel classrooms at seven various DepEd elementary schools in Cagayan de Oro City. About half of these mosquito traps were installed in classrooms with Olyset® nets, while the other half were installed in classrooms without Olyset® nets. After requesting entry from the school principal and teachers, the traps were installed at corners inside the classrooms for about two months. Sampling was done twice in each sentinel classroom throughout the surveillance period.

From mosquito trap surveillance in 70 elementary classrooms, 1452 adult mosquitoes and 806 larvae were caught in the mosquito traps. A mean capture of 4.9 adult *Aedes* mosquito/trap was calculated from classrooms with Olyset® nets in contrast to a mean of 10.2 adult *Aedes* mosquito/trap in classrooms without Olyset® nets (Fig. 4).

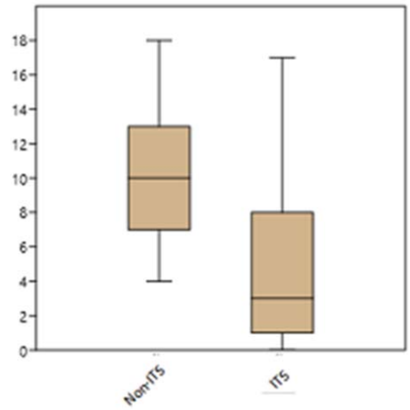

Fig. 4 Comparison of means of *Aedes* female counts between classrooms with and without Olyset® nets.

Test of equal means detected highly significant differences in mosquito capture between classrooms with and without Olyset® nets ($p=8.82x10^{-06}<0.05$). Larval capture in the traps also showed substantial differences between classrooms with and without Olyset® nets ($p=7.5x10^{-05}<0.05$) (Fig. 5). The breakdown of female and male *Aedes* mosquitoes and non-dengue mosquitoes are listed in Table 3.

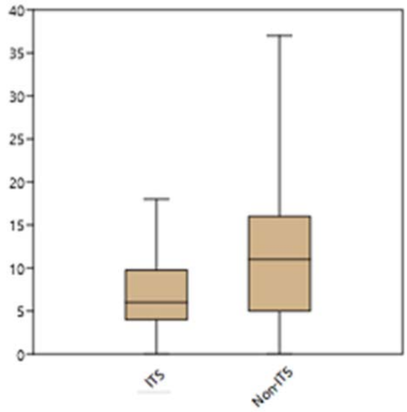

Fig. 5 Comparison of means of *Aedes* larvae counts between ITS and non-ITS classrooms

Table 2 Counts of adult *Ae.aegypti*, *Ae.* albopictus and non-dengue mosquitoes caught during mosquito trap suveillance in DepEd classrooms.

	Ae. aegypti		*Ae. albopictus*		Non-target Mosquitoes	
	Female	male	Female	male	Female	male
With Olyset®	176	31	14	14	257	83
Without Olyset®	289	57	22	7	382	120

A discrepancy was also noticed between *Ae. aegypti* and *Ae. albopictus* capture in the traps ($p=1.1x10^{-17}<0.05$). There were two likely reasons for this difference in capture. One reason may be based on their blood-feeding habits. It was being considered zoophilic by feeding habit, *Ae. albopictus* tend to forage outdoors where various blood sources exist. In contrast, *Ae. aegypti,* is anthropophilic. It usually forages indoors, where its most preferred blood source is present (Delatte et al., 2010; Bonizzoni et al., 2013; Sullivan et al., 1997; Harrington et al., 2001; Ponlawatt and Harrington, 2005; Richards et al., 2006). The second reason for the contrasting trap captures may be their breeding preferences. *Ae. aegypti* prefers clear and less polluted waters in domestic containers inside or near human dwellings. *Ae. albopictus* prefers natural containers or outdoor artificial habitats containing organic debris (Chareonviriyaphap et al.,2003; Dom et al., 2013). The water in the indoor mosquito traps was devoid of organic debris, so more *Ae. aegypti* were captured in the mosquito traps inside the classrooms than *Ae. albopictus.* Based on mosquito trap capture, *Aedes* adult females and larval abundances were significantly low in classrooms with Olyset® nets than in classrooms without Olyset® nets ($p=1.1x10^{-17}<0.05$)(Table 4). This was unexpected since it was observed that many of the Olyset® nets need replacement due to the presence of holes and tears. Furthermore, many ITS classrooms' lack of screened doors made the intervention appear compromised. It was suspected that the mosquitocidal impact of Olyset® nets had already been significantly reduced. Many classrooms with Olyset® nets did not even have screen doors. And the doors were left open during classes because, according to teachers, air ventilation was severely limited by Olyset® nets. This may have allowed unimpeded entry or movement of *Aedes* mosquitoes and other non-target mosquitoes between classrooms (Fig 6). It has also been observed that many of the Olyset® nets, especially those with 1-year-old usage already had tears and holes of various sizes. This status should have been attended by school authorities but was not because of many other concerns in the school.

Table 3 Means and abundance of *Aedes* adult females and larvae in classrooms with and without Olyset® nets based on count in mosquito traps

	Classroom with Olyset® nets (ITS)	A classroom without Olyset® nets (Non-ITS)
Mean of adult female *Ae.*	4.97+4.97	10.22+0.67
Sum of Adult female *Ae.*	174	358
Mean of *Ae.*larvae	6.66+0.73	12.30+1.30
Sum of *Ae.* larvae	240	566

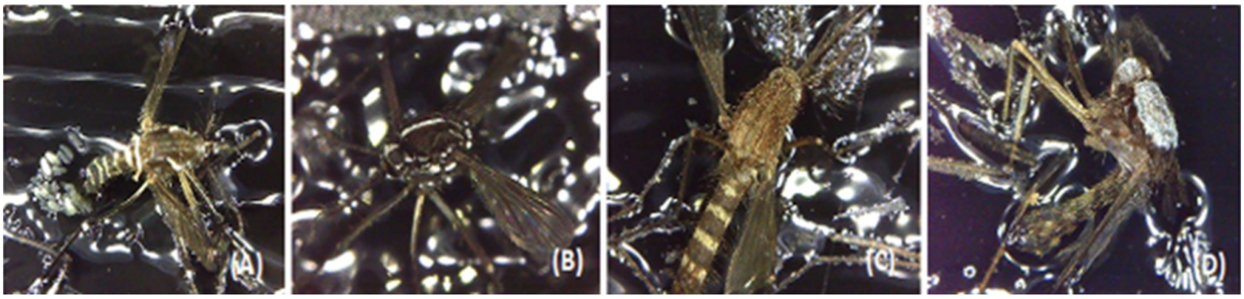

Fig. 6 Samples of adult mosquitoes caught in mosquito adult-larval traps: (A) *Ae. aegypti* (B) *Ae. albopictus*, (C) *Culex quinquefasciatus*, (D) *Cx. gelidus*

4 Summary and Conclusion

This study set out to conduct an entomological evaluation of Olyset® nets that have been installed in DepEd elementary classrooms. The methods used were laboratory-based WHO Cone bioassay and field surveillance of adult female *Aedes* and larval abundance in selected DepEd classrooms using mosquito adult-larval traps.

Based on WHO 98-100% insecticide susceptibility guidelines, the cone bioassay of *Ae.* results. *Aegypti* and *Ae. albopictus* on Olyset® nets showed these mosquitoes were not fully susceptible to 2% permethrin in the Olyset® nets. Therefore, the Olyset® nets are not fully effective in producing the desired 98-100% knockdown and mortality for intervention against dengue mosquitoes.

Mosquito adult-larval trap surveillance on *Ae. aegypti* and *Ae. albopictus* in seventy DepEd elementary classrooms showed significantly low mean capture of adult and larvae of dengue vector mosquitoes in classrooms with Olyset® nets than in classrooms without Olyset® nets. Mean capture of 4.9 adult female *Aedes* mosquitoes per trap ere obtained in classrooms with Olyset® nets, compared to 10.2 *Aedes* mosquitoes/trap caughtin classrooms without Olyset® nets. Test of equal means detected highly significant differences in mosquito capture between classrooms with and without Olyset® nets ($p=8.82x10^{-06}<0.05$). Larval capture in the traps equally showed substantial differences between classrooms with and without Olyset® nets ($p=7.5x10^{-05}<0.05$).

Nevertheless, mosquito adult-larval trap surveillance showed that the Olyset® nets are ineffective as an intervention against *Ae. aegypti* and *Ae. albopictus,* because vector mosquitoes are still present with Olyset® nets in classrooms. If the Olyset® nets were practical, no *Aedes* mosquitoes or very few mosquitoes would have been present. But the results show that they are still a clear and present danger in the classrooms with Olyset® nets.

Furthermore, the lack of complete enclosures in the classrooms, like screen doors, has unfortunately compromised the efficacy of the intervention. Therefore, the material integrity of the Olyset® nets and other accessories needed to maintain the classroom enclosure against dengue vectors should be assessed and attended to sustain the intervention. It would be interesting to follow up on the reductive impact of Olyset® nets against the hole index in the nets. Furthermore, a Cone bioassay on *Ae* populations over a wider area with GIS mapping of percent mortality and knockdown would begin a comprehensive study of pyrethroid resistance in the *Aedes* mosquitoes.

Acknowledgment

The authors would like to thank the Regional Office of the Department of Education-Region X, the Division Office, and School Principals of Cagayan de Oro City Division (DepEd) for the approval and support in the conduct of this study. The authors wish to thank the Municipal Health Workers of the Department of Health-Region X Office, the Sanitary Health Workers of the City Health Office of Cagayan de Oro City for their assistance and support in conducting this study, and the Premier Research Institute of Science and Mathematics (PRISM) of the MSU-Iligan Institute of Technology (MSU-IIT) for the partial support in the conduct of this study.

References

Agrupis KA, Ylade M, Aldaba J, Lopez AL, Deen J. 2019. Trends in dengue research in the Philippines: A systematic review. PLoSNegl Tropical Diseases, 13(4): e0007280

Ahmad-Azri M, Syamsa RA, Ahmad-Firdaus MS, Aishah-Hani A. 2019. A comparison of different types of ovitraps for outdoor monitoring of *Aedes* mosquitoes in Kuala Lumpur. Tropical Biomedicine, 36(2): 335-347

Autry D, Dixon D, Bibbs CS, Khater EIM, Xue ARD. 2021. Field comparison of autocidal gravid ovitraps and in2care traps against *Aedes aegypti* in downtown Saint Augustine, Northeastern Florida. Journal of the Florida Mosquito Control Association, 68: 92-96

Barrera R, Amador M, Acevedo V, Hemme RR, Félix G. 2014. Sustained, area-wide control of *Aedes aegypti* using CDC autocidal gravid ovitraps. American Journal of Tropical Medicine and Hygiene, 91(6): 1269-1276

Bonizzoni M, Gasperi G, Chen X, James AA. 2013. The invasive mosquito species *Aedes albopictus*: Current knowledge and future perspectives. Trends in Parasitology, 29(9): 460-468

Cevallos V, Ponce P, Waggoner J, Pinsky BA, Coloma J, Quiroga C, Morales D, Cardenas MJ. 2017. Zika and Chikungunya virus detection in naturally infected *Ae. aegypti* in Ecuador. Acta Tropica, 177: 74-80

Chareonviriyaphap T, Akratanakul P, Nettanomsak S, Huntamai S. 2003. Larval habitats and distribution patterns of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse), in Thailand. Southeast Asian Journal Tropical Medical Public Health, 34: 529-535

Chaves LF, Friberg MD. 2021. *Aedes albopictus* and *Aedes flavopictus* (Diptera: Culicidae) pre-imaginal abundance patterns are asso-ciated with different environmental factors along an altitudinal gradient. Current Research in Insect Science, 1

Cheng ML, Ho BH, Barnett RE, Goodwin N. 1982. Role of a modified ovitrap in the control of *Aedes aegypti* in Houston, Texas, USA. Bulletin of World Health Organization, 60: 291-296

De Las Llagas LA, Medina A. 2002. A Final Report of A Collaborative Study on Establishment of Sensitive Vector Indicator for Dengue Surveillance. Essential National Research Terminal Report. Ministry of Health, Manila, Philippines

Delatte H, Desvars A, Bouetard A, Bord A, Gimonneau G, Vourc'h G, Fontenille D. 2010. Blood-feeding behavior of *Aedes albopictus*, a vector of Chikungunya on La Reunion. Vector-Borne and Zoonotic Diseases, 10(3): 249-258

Dev V, Raghavendra K, Barman S, Phookan S, Dash AP. 2010. Wash-resistance and efficacy of Olyset® net, a permethrin-incorporated long-lasting insecticidal netting, against *Anopheles minimus*-transmitted Malaria in Assam, Northeastern India. Vector-Borne and Zoonotic Diseases, 10(4): 403-410

Dom NC, Abmad AH, Ismail R. 2013. Habitat characterization of *Ae.* spp. Breeding in urban hotspot area. Procedia Social and Behavioral Sciences, 85: 100-109

Fay RW, Eliason DA. 1966. A preferred oviposition site as a surveillance method for *Aedes aegypti*. Mosquito News, 26: 531-535

Focks DA. 2003. A review of entomological sampling methods and indicator for dengue vectors. Gainesville: World Health Organization,Swizerland

Garcia-Luna SM, Chaves LF, Juarez JG, Bolling BG, Rodriguez A, Presas YE, Mutebi JP, Weaver SC, Badillo-Vargas IE, Hamer GL, Qualls WA. 2019. From surveillance to control: Evaluation of a larvicide intervention against *Aedes aegypti* in Brownsville, Texas. Journal of the American Mosquito Control Association, 35(3): 233-237

Gualberto DA, Demayo CG. 2022a. Laboratory and Field Evaluation of an innovated adult-larval mosquito trap for the capture of dengue vector mosquitoes. International Journal of Mosquito Research. 9(2): 44-50

Gualberto DA, Demayo CG. 2022b. Evaluation of the efficacy of pyrethroid pesticides against selected strains of *Aedes* mosquitoes using the who susceptibility test and by thermal fogging. International Journal of Mosquito Research, 9(2): 58-64

Gunning C, OkamotoKW, Astete H, Vasquez G., Erhardt E, Del Aguila C, Pinedo R, Cardenas R, Pacheco C, Chalco E, Rodriguez-Ferruci H, Scott TW, Lloyd AL, Gould F, Morrison AC. 2018. Efficacy of *Aedes aegypti* control by indoor Ultra Low Volume (ULV) insecticide spraying in Iquitos, Peru. Plos Neglected Tropical Diseases, 12(4): e0006378

Harrington LC, Edman JD, Scott TW. 2001. Why do female *Aedes aegypti* (Diptera: Culicidae) feed preferentially and frequently on human blood? Journal of Medical Entomology, 38(3): 411-422

Higgs S, D Vanlandingham D. 2015. Chikungunya virus and its mosquito vectors. Vector-Borne and Zoonotic Diseases, 15: 231-240,.

Igarashi A. 1997. Impact of dengue virus infection and its control. FEMS Immunology and Medical Microbiology, 18(4): 291-300

Johnson, BJ, Ritchie SA, Fonseca DM. 2017. The state of the art of lethal oviposition trap-based mass interventions for arboviral control. Insects, 7: 5

Juarez JG, Chaves LF, Garcia-Luna SM, Martin E, Badillo-Vargas I, Medeiros MCI, Hamer GL. 2021. Variable coverage in an Autocidal Gravid Ovitrap intervention impacts the efficacy of *Aedes aegypti* control. Journal of Applied Ecology, 58: 2075-2086.

Khater EIM, Autry D, Gaines MK, Xue ARD. 2022. Field Evaluation of autocidal gravid ovitraps and in2care traps against *Aedes* mosquitoes in Saint Augustine, Northeastern Florida. Journal of the Florida Mosquito Control Association, 69: 48-54

Lambrechts L, Scott TW, Gubler D. 2010. Consequences of the expanding global distribution of *Aedes albopictus* for dengue virus transmission. PLoS Neglected Tropical Diseases, 4: e646

Lee HL. 1992. *Aedes*ovitrap and larval surveys in several suburban communities in Selangor, Malaysia. Mosquito-Borne Diseases, 9: 15

Leisnham PT, LaDeau SL, Juliano SA. 2014. Spatial and temporal habitat segregation of mosquitoes in urban Florida. PLoS ONE, 9(3): e91655

Lenhart A, Morrison AC, Paz-Soldan VA, Forshey BM, Cordova-Lopez JJ, Astete H, Elder JP, Sihuincha M, Gotlieb EE, Halsey ES, Kochel TJ, Scott TW, Alexander N, McCall PJ. 2020. The impact of insecticide-treated curtains on dengue virus transmission: A cluster-randomized trial in Iquitos, Peru. PLoS Neglected Tropical Diseases, 14(4): 1-17

Lenhart AE, Walle M, Cedillo H, Kroeger A. 2005. Building a better ovitrap for detecting *Aedes aegypti* oviposition. Acta Tropica, 96: 56-59

Lourenço-de-Oliveira R, Lima JBP, Peres R, Alves FDC, Eiras AE, Codeço CT. 2008. Comparison of different uses of adult traps and ovitraps for assessing dengue vector infestation in endemic areas. Journal of the American Mosquito Control Association, 24(3): 387-392

Mackay AJ, Amador M, Barrera R. 2013. An improved autocidal gravid ovitrap for the control and surveillance of *Aedes aegypti*. Parasites and Vectors, 6(1)

Martin E, Medeiros MCI, Carbajal E, Valdez E, Juarez JG, Garcia- Luna S, Salazar A, Qualls WA, Hinojosa S, Borucki MK, Manley H., Badillo-Vargas IE, Frank M, Hamer GL. 2019. Surveillance of *Aedes aegypti* indoors and outdoors using Autocidal Gravid Ovitraps in South Texas during local transmission of Zika virus, 2016 to 2018. Acta Tropica, 192 (January): 129-137

N'Guessan R, Darriet F, Doannio JM, Chandre F, Carnevale P.2001. Olyset® net efficacy against pyrethroid–resistant *Anopheles gambiae* and *Culex quinquefasciatus* after 3 years' field use in Côte d'Ivoire. Medical and Veterinary Entomology, 15: 97-104

Nguyen T, Hoang VT, Tran CT, Nguyen UN, Truong T, Hoa T. 1996. The effect of Olyset® Net screen to control the vector of dengue fever in Vietnam. Dengue Bulletin, 20: 87-92.

Obenauer PJ, Kaufman P, Allan SA, Kline DL. 2009. Vector-Borne Diseases, Surveillance, Prevention. 2009. Infusion-baited ovitraps to survey ovipositional height preferences of container-inhabiting mosquitoes in two Florida Habitats. Journal of Medical Entomology, 46(6): 1507-1513

Obregón JA, Ximenez MA, Villalobos EE, de Valdez MRW. 2019. Vector mosquito surveillance using centers for disease control and prevention autocidal gravid ovitraps in San Antonio, Texas. Journal of the American Mosquito Control Association, 35(3): 178-185

Okuno T, Ito T, 2006. Development of 'Olyset®' Net as a Tool for Malaria Control. Sumitomo Kagaku R & D Report, 2

Owusu HF, Jančáryová D, Malone D, Müller P. 2015. Comparability between insecticide resistance bioassays for mosquito vectors: time to review current methodology? Parasites and Vectors, 8: 357

Ponlawatt A, Harrington LC. 2005. Blood feeding patterns of *Aedes. aegypti* and *Aedes albopictus* in Thailand.

Journal of Medical Entomology, 42(5): 844-849

Powell JR. 2018. Mosquito-Borne Human Viral Diseases: Why *Aedes aegypti*? American Journal of Tropical Medicine and Hygiene, 98(6): 1563-1565.

Rawlins SC, Martinez R, Wiltshire S, Legall G. 1998. A comparison of surveillance systems for the dengue vector *Aedes aegypti* in Port of Spain, Trinidad. Journal of the American Mosquito Control Association, 14: 131-136

Regis L, Monteiro AM, De Melo-Santos MAJ, de Oliveira CMF, Correia JC, de Albuquerque CMR. 2008. Developing new approaches for detecting and preventing *Aedes aegypti* population outbreaks: basis for surveillance, alert and control system. Memórias do Instituto Oswaldo Cruz, 103: 50-59

Richards SL, Ponnusamy L, Unnaschi TR, Hassan HK, Apperson CS, 2006. Host-feeding patterns of *Aedes albopictus* (Diptera: Culicidae) in relation to the availability of human and domestic animals in suburban landscapes of Central North Carolina. Journal of Medical Entomology, 43(3): 543-551

Ritchie SA, Long S, Hart A, Webb CE, Russell RC. 2003. An adulticidal sticky ovitrap for sampling container-breeding mosquitoes. Journal of the American Mosquito Control Association, 19(3): 235-242

Ritchie SA. 2014. Dengue vector bionomics: why *Aedes aegypti* is such a good vector. In: Dengue and Dengue Hemorrhagic Fever (2nd ed) Gubler D, ed). 455-480, CAB International, Oxfordshire, UK

Savage HM, Smith GC. 1994. Identification of damaged adult female specimens of *Aedes aegypti* and *Aedes* albopictus in the new world. Journal of the American Mosquito Control Association, 10(3): 440-442

Sharp TM, Lorenzi O, Torres-Velásquez B, Acevedo V, Pérez- Padilla J, Rivera A, Muñoz-Jordán, J, Margolis HS, Waterman SH, Biggerstaff B J, Paz-Bailey G, Barrera R. 2019. Autocidal gravid ovitraps protect humans from chikungunya virus infection by reducing Aedes aegypti mosquito populations. PLoS Neglected Tropical Diseases, 13(7)

Silver JB. 2013. Mosquito ecology: Field sampling methods. In: Mosquito Ecology: Field Sampling Methods (3rd ed, Vol 53). Springer Science+Business+Media

Sullivan MF, Gould DJ, Maneechai S. 1997. Observations on the host range and feeding preferences of *Aedes albopictus* (Skuse). Journal of Medical Entomology, 8(6): 713-716

Tami A, Mubyazi G, Talbert A, Mshinda H, Duchon S, Lengeler C. 2004. Evaluation of Olyset® insecticide-treated nets distributed seven years previously in Tanzania. Malaria Journal, 3(19): 19

Tsunoda T, Kawada H, Huynh TT, Luu LL, Le SH, Tran HN, Vu HT, Hasebe F, Tsuzuki A, Takagi M. 2013. Field trial on a novel control method for the dengue vector, *Aedes aegypti*, by the systematic use of Olyset® Net and pyriproxyfen in Southern Vietnam. Parasit Vectors: Jan11, 6:6 doi: 10.1186/1756-6-6.

Vythilingam I, Pascua BP, Mahadevan S. 1996. Assessment of a new type of Permethrin impregnated mosquito net. Journal of Bioscience, 7(1): 63-70

Wan Norafikah O, Nazni WA, Noramiza S, Shafa'ar-Ko'ohar S. 2011. Ovitrap surveillance and mixed infestation of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) in northern region and southern region of Malaysia. Health Environ Journal, 2: 1-5

WHO. 2001. Review of Olyset® Net in Report of the fifth WHOPES Working Group Meeting WHO/HQ. Geneva, Swizerlands

WHO. 2012. Global Strategy For Dengue Prevention and Control 2012-2020. Rome, Italy

WHO. 2013. Guidelines for laboratory and field-testing of long-lasting insecticidal nets. WHO Pesticide Evaluation Schemes. WHO/HTM/NTD/WHOPES/2013.1, Rome, Italy

World Health Organization-Regional Office for the Western Pacific (WHO-WPRO). 1995. Western Pacific education in action series No. 8: Guidelines for dengue surveillance and mosquito control. WHO WPRO, Manila, Philippines